THE

JOURNAL OF EXPERIMENTAL MEDICINE

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

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AND

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PUBLISHED FOR

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK

VOLUME FOURTEENTH

WITH FIFTY-THREE PLATES AND TWENTY-FIVE FIGURES IN THE TEXT

THE NEW ERA PRINTING COMPANY

1911

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

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STRONGYLOIDES INFECTIONS IN MAN AND ANIMALS IN THE ISTHMIAN CANAL ZONE.*

By S. T. DARLING, M.D.

(*From the Ancon Hospital, Ancon, Canal Zone.*)

PLATES 1 TO 3.

Most of the published accounts of infections by Strongyloides stercoralis have been based on cases studied or on material obtained in temperate zones. This paper contains the results of observations on the incidence of infection among some of the inhabitants of the Isthmian Canal Zone (Hospital and Asylum cases); also the results of a study of cultures and of tissue infected with purely tropical strains of three species of strongyloides: S. stercoralis, S. cebus, and S. nasua. The heterogamous mode of development, the influence of external factors on viability and growth, and the relation of strongyloides infections to diarrhea, anemia, and bacterial infections of the intestinal tract have been inquired into.

The species studied are: S. stercoralis, stools and autopsy material from West Indian negroes, native Colombians, and Spanish laborers; S. cebus from several individuals of *Cebus hypoleucus*, the white-faced organ grinder monkey; and S. nasua from two specimens of the ant bear, *Nasua nasica Panamensis*.

STRONGYLOIDES STERCORALIS.

DESCRIPTION, LIFE HISTORY, AND MODES OF INFECTION.

Description.—Strongyloides stercoralis[1] (Stiles and Hassall, 1902) was discovered in 1876 by Normand in stools and at autopsy in French soldiers from Cochin China. The nematodes described as

* Received for publication, March 8, 1911.

[1] Synonomy: Anguillula intestinalis and A. stercoralis, Bavay, 1877; Leptodera stercoralis and L. intestinalis, Cobb, 1879; Pseudorhabditis stercoralis, Perroncito, 1881; Rhabdonema strongyloides, Leuckart, 1883; Strongyloides intestinalis, Grassi, 1883; Rhabdonema intestinalis, Blanchard, 1885.

two species by Bavay[2] under the name Anguillula intestinalis, the parasitic generation, and Anguillula stercoralis, the free-living generation, were demonstrated by Leuckart as succeeding generations of one species.[3]

Anguillula intestinalis, the parasitic generation, regarded by Leuckart as hermaphroditic, and by Rovelli as a female reproducing by parthenogenesis, is a small nematode about 2.4 mm. long and 0.045 to 0.048 mm. wide. The esophagus is long and cylindrical with a narrow central canal and occupies the anterior fourth of the body. The posterior end is pointed and the anus opens in front of the posterior tip. The vulva is in the posterior third of the body, and the ovary generally contains about five segmented or unsegmented ova which measure 0.046 to 0.048 mm. in cross section. The free-living generation, Anguillula stercoralis, is derived from the eggs laid by A. intestinalis in the epithelium of the villi and crypts of the small intestine. This generation is sexually differentiated. Its body is cylindrical and smooth, with a slender anterior extremity and a pointed tail. The mouth has four lips. The esophagus is short with a double dilatation, and there are three chitinous teeth in the posterior dilatation. The anus opens in front of the tail end. The males measure 0.7 mm. in length and 0.035 mm. in width. They carry their posterior end curled up. The two spicules are small, short, and somewhat curved. The females measure 1 mm. in length and about 0.05 mm. in width. The tail end is straight and pointed. The vulva lies behind the middle of the body. The ova measure 0.07 by 0.045 mm. (after Braun). The following table shows the variation in size of worms from the Panama cases.

Life History.—The current conception of the life history of S. stercoralis is as follows:—

The parthenogenetic female mother worm deposits her ova in the epithelial cells lining the crypts and covering the villi of the small intestine. When the embyros mature, they escape into the lumen of the bowel as rhabditiform larvæ and are usually evacuated as such. These larvæ in moist soil of proper temperature develop either, on the one hand, into filariform larvæ, which, upon ingestion, or infection through the skin, reach the intestinal wall, becoming female mother worms, and thus complete the so-called "short circuit" or direct phase of their development; or, on the other hand, these rhabditiform larvæ develop into the sexually differentiated free-living generation, the rhabditiform larvæ of which all develop into filariform larvæ to become female mother worms, thus completing the indirect phase. Most observers are of the opinion that the direct mode of development is more common in colder regions, while the indirect mode prevails in tropical climates. It is also believed that female mother worms may be derived from larvæ which never leave the intestinal tract of the host, thus producing reinfection or auto-infection.

[2] Bavay, *Compt. rend. Acad. d. sc.*, 1876, lxxxiii, 694; 1877, lxxxiv, 266.

[3] An excellent account of the various contributions to this subject, with a good bibliography up to 1901, is that of Thayer, *Jour. Exper. Med.*, 1901–05, vi, 75.

TABLE I.

Measurements of Strongyloides stercoralis from the Panama Cases.

	Length in mm.	Breadth in mm.	Mouth to base of esophagus in mm.	Mouth to to vulva in mm.	Mouth to anus in mm.	Anus to posterior tip in mm.	Ova in mm.
Female mother worm.....	2.640	0.045	0.720				0.036–0.060
Rhabditiform larvæ of female mother worm.....	0.285— 0.320	0.021— 0.018					
Filariform larvæ of female mother worm.....	0.440— 0.480— 0.608— 0.512	0.016— 0.017— 0.018					
Free-living form: males..	0.800— 0.816— 0.960— 1.260	0.040— 0.048— 0.072	0.072 0.152		1.156		
Free-living form: females Bred in refrigerator, temperature 67°–72° F.....	1.392— 1.488— 1.680 1.760—	0.080	0.140	0.717	1.257	0.135	
	1.264— 1.344— 1.440— 1.504— 1.648			0.640— 0.704— 0.784 0.784 0.864	Vulva to tip of tail 0.624 0.640 0.656 0.720 0.784		

The Mode of Infection.—Wilms[4] and Looss[5] succeeded in infecting man with strongyloides larvæ by the mouth and skin respectively, while Van Durme,[6] Marzocchi,[7] and Gonder[8] have demonstrated that filariform larvæ can penetrate the integument of animals and sometimes cause infection in this way.

I have successfully infected *Cebus* with filariform larvæ of S. cebus by mouth. A number of cross-inoculations or feeding experiments failed; for example, S. stercoralis, filariform indirect phase larvæ, did not infect *Cebus* through the skin. Guinea pigs could not be infected by mouth with S. stercoralis with either direct or indirect phase larvæ, and S. nasua, indirect phase larvæ, when fed to rabbits did not produce an infection.

[4] Wilms, quoted by Leichtenstern, *Deutsch. med. Wchnschr.*, 1898, xxiv, 118.
[5] Looss, *Compt. rend. VI Cong. internat. de Zool.*, 1905, 225.
[6] Van Durme, *Thompson Yates Laboratories Report*, 1902, iv, 471.
[7] Marzocchi, *Gior. d. r. Accad. di med. di Torino*, 1907, series 4, xiii, 3.
[8] Gonder, *Arb. a. d. k. Gsndhtsamte*, 1907, xxv, 485.

LOCALIZATION OF STRONGYLOIDES STERCORALIS AND OTHER ENTOZOA IN THE INTESTINAL TRACT.

A study of the relation of S. stercoralis and various entozoa to their respective hosts with regard to their localization in the bowel has brought out some interesting facts. First, to note briefly the incidence of intestinal parasites in man in this region, the following table is taken from the Chief Sanitary Officer's Annual Reports in which are tabulated the results of stool examinations at the Commission Hospitals. The examinations represent nearly all the medical patients, who are made up of West Indian negroes, native Panamans, or Colombians, and a number of white European laborers from Spain, Italy, and Greece.

	1907.	1908.	1909.
Number of stool examinations....	20,773	16,423	7,667
Variety of parasite or ovum found:			
Roundworm ova.....	1,873	972	370
Hookworm ova.....	3,340	2,396	1,358
Whipworm ova.....	3,931	2,812	999
Strongyloides larvæ.....	698 (3%)	643 (3.9%)	229 (2.9%)
Bilharzia ova.....	62	19	12
Tapeworm ova.....	16	33	11
Oxyuris vermicularis.....	2	8	0
Balantidium coli.....	22	20	3

By means of cultural methods, a much higher degree of parasitization may be detected than that noted by the usual cover-slip method of examination as given in the above table. In the Insane Division, I recently determined by special methods of examination that:—

18 per cent. of all patients were infected with strongyloides.
20 per cent. of the native patients were infected with strongyloides.
6.4 per cent. of the non-native patients were infected with strongyloides.
21.3 per cent. of the male patients were infected with strongyloides.
14.9 per cent. of the female patients were infected with strongyloides.
28.5 per cent. of the very dirty patients were infected with strongyloides.
31.7 per cent. of the patients over 50 years were infected with strongyloides.

Each of the parasites selects a particular region of the intestinal tract for its field of action and is rarely found away from it. Ascaris lumbricoides, Agchylostoma duodenale, and Necator americanus

prefer the jejunum and ileum. The two latter are never found attached to the mucosa of the colon, stomach, or esophagus, and are but rarely attached to the mucosa of the duodenum or pylorus, except in very severe infections. Trichuris trichiura is most commonly found in the cecum, occasionally in the vermiform appendix or other parts of the large bowel, but it is never attached to the mucosa of the small intestine. This is so constant that I was enabled on one occasion to conclude from an examination of sputum, which contained fecal matter and trichuris ova, that there was a communication between the large bowel and a bronchus. At autopsy a few days later, a fistulous tract was found between the splenic flexure of the colon and the bronchioles in a gangrenous area of the lower lobe of the left lung. The commonest location of the ulcers in amebic colitis is the cecum—the habitat of the whipworm—suggesting that the points of attachment of the latter serve as portals of entry for amebæ. Schistosomum hematobium, Balantidium coli, and Oxyuris vermicularis are always found when present in the large bowel. In a recent case of balantidic dysentery, the parasites at autopsy were found only in the vermiform appendix. Strongyloides stercoralis, the female mother worm, is found only in the upper parts of the small intestine. One of my autopsies showing a moderately heavy infection yielded the following notes on the location and relative numbers of female mother worms and embryos from smears taken at different parts:—

Stomach, none. Duodenum (2 cm. outside pyloric ring), about 40 rhabditiform embryos; first half, two rhabditiform embryos; second half, one rhabditiform embryo. Jejunum, first third, four embryos; second third, 30 embryos and two female mother worms; third third, about 30 embryos. Junction of jejunum with ileum about 25 embryos and one female mother worm. Ileum, only one rhabditiform embryo found. I have never found a female mother worm or imbedded embryos in the colon in any of the cases that have come to autopsy.

The localization of the intestinal parasites of man and animals depends on conditions apparently affecting the structure or the physiological secretions of the various tracts. Some anatomical peculiarity of the mucosa or the nature and reaction of the food supply and surrounding chyme may influence the point of attach-

ment or location of the different parasites. In order to test the possible effect of the chemical reaction of the chyme on the localization of intestinal parasites, the reaction of the contents of the stomach, jejunum, and cecum was determined in several patients an hour or so after death, but without definite results. A comparative study of the bionomics of helminths indicates that certain species usually select homologous portions of the intestinal tracts in different animals. For example, sclerostoma and trichuris select the large bowel of horses, sheep, antelopes, cattle, goats, etc. Hookworms (necator, agchylostoma, trigonocephala, etc.) select the jejunum and ileum of man, dogs, goats, cattle, etc. Ascarides select the small bowel or stomach. It was from these and analogous facts that Manson was led to formulate his hypothesis of the collateral descent of hosts and their parasites, as a corollary to which it might be suggested that the localization of a parasite in its host depends also on inheritance.

THE RELATION OF STRONGYLOIDES INFECTIONS TO DIARRHEA, ANEMIA, AND BACTERIAL INFECTIONS OF THE INTESTINAL TRACT.

Although formerly it was the opinion of many that infections by S. stercoralis were the cause of Cochin China and other forms of diarrhea, the opinion has been gaining ground in recent years that these infections are of little consequence. For the purpose of ascertaining whether any clinical importance should be attached to this infection, an analysis was made of 121 consecutive cases occurring at Ancon Hospital during the year ending June 30, 1910, and of 54 cases in the Insane Division. The 121 cases were distributed as follows:—

This represents approximately an even distribution of the infection among all the laborers, white and black. Most of the patients were admitted for malaria, so that the strongyloides were incidentally found as a result of the routine stool examinations. Only three cases were diagnosed outright as strongyloidosis. Nine of the 121 cases were diagnosed as diarrhea or dysentery, or fell into the class of diarrheal diseases, and of these nine, most of the patients had mucous or bloody stools and tenesmus, clearly due to colitis

White.		Black.	
Greece	2	Barbados	24
Italy	4	Jamaica	17
Spain	17	Martinique	10
Portugal	2	Panama	3
Panama	1	Monserrat	2
Peru	1	Colombia	4
Colombia	2	Guadaloupe	3
Nicaragua	1	Trinidad	1
France	1	St. Vincent	3
United States	2	British Guiana	3
	33	Grenada	4
		Mexico	4
		Antigua	4
		St. Lucia	2
		Dominica	2
		Not known	2
			88

and not to the strongyloides present, for the latter do not invade the mucosa of the large bowel. Many of the patients stated that their bowels were normal, and as they remained in the hospital for periods up to thirty-five days, with an average of 14.9 days, it was possible to learn whether there was an excessive number of bowel movements. Sixty-eight cases were especially analyzed with regard to this point.

Six	patients	had	an	average	of	1 to 2	stools	per day.
Two	patients	had	an	average	of	2	stools	per day.
Twenty-one	patients	had	an	average	of	2 to 3	stools	per day.
Nine	patients	had	an	average	of	3	stools	per day.
Twenty	patients	had	an	average	of	3 to 4	stools	per day.
Four	patients	had	an	average	of	4	stools	per day.
Five	patients	had	an	average	of	4 to 5	stools	per day.
One	patient	had	an	average	of	5 to 6	stools	per day.

All these patients received a purge on entrance, and many received thymol treatment once or twice. These are all included in the calculation of the average number of stools, the number of which in this series was about the same as those noted in cases of malaria associated with hookworms in which one or two thymol treatments had been administered. Of 54 cases of strongyloidosis detected by cultural methods among the insane patients who have been under observation for periods up to three or four years, none had been

subject to diarrhea. The stools of monkeys and ant bears infected with strongyloides were usually well formed and there was never any indication of diarrhea.

It would appear, then, that S. stercoralis can not be incriminated as a causative agent in diarrhea, for none of the patients harboring them appeared to suffer any inconvenience by their presence.

Anemia.—A study of sections of infected tissue from the duodenum and jejunum shows slight but very definite pathological changes, and while it is suspected that these proliferative and exudative changes in the mucosa may be associated with some anemia, the amount would be impossible to determine on account of the frequently associated malaria or uncinariasis. Examination of the blood of monkeys and ant bears in every case showed some degree of anemia, but careful search at autopsy in the ant bear, having the highest degree of anemia, failed to reveal any other cause than the strongyloides infection. The first one to describe the peculiar habits of this nematode of burrowing into the mucosa and of causing pathological changes was Askanazy,[9] who has shown that the mother worm invades the mucosa to deposit her ova and to obtain food there.

In sections of the duodenum and jejunum from one of my autopsies in which there was a very heavy infection, the interglandular stroma is here and there infiltrated with round and plasma cells. The supporting reticulum of Brunner's glands also contains collections of plasma cells and there is an increased number of polymorphonuclear leucocytes, both neutrophilic and eosinophilic, associated with the round and plasma cells.

Marchi Preparations.—The embryos imbedded in the epithelium of the crypts generally stain a little more deeply than the surrounding tissues. This is due largely to the presence of large, black granules of irregular size scattered through the embryos, 4, 5, and 6 microns in diameter. They are also found in the larger embryos which are escaping from the crypts, and in one longitudinal section of an adult mother worm, there are several fat droplets in the intestinal tube. The segmented ova encysted in the epithelium of the crypts stain more deeply than the surrounding epithelium and they contain spherical and biscuit-shaped globules of fat beneath their capsules. By careful focusing, a few fat droplets can be seen in the interior of the embryos. One can not escape the impression from these preparations that the fat droplets seen in the ova,

[9] Askanazy, *Centralbl. f. Bakt. u. Parasitenk., 1te Abt.*, 1900, xxvii, 569.

embryos, and adult worms are derived as fat from the chyle of the host. This confirms Askanazy's findings and bears out his statement that the mother worm burrows into the mucosa for food.

Duodenum No. 1.—The peritoneum and muscle wall are normal. The villi and crypts show a very heavy infection by strongyloides. Many ova and larvæ are seen in all parts of the mucous membrane. The ova are found between the epithelial cells of the crypts or villi or between the epithelial cells and basement membrane, pushing the cells to one side and altering the outline of the crypt or villus (figure 1). The embryos are seen in all stages of development, from unsegmented ova to the completely developed larvæ. Free larvæ are occasionally seen in the interglandular lymphoid tissue and in the lymphoid follicles of the submucous coat. Occasionally a female mother worm can be seen with her cephalic end deeply placed in a crypt or penetrating the mucosa (figures 1 and 2). The crypts frequently contain extruded larvæ on their way to the lumen of the bowel (figure 3).

Duodenum No. 2.—This presents the same picture as No. 1. On the margin of a Brunner's gland there are several cross and oblique sections of a female mother worm. At this point there is a break in the muscularis mucosæ and an attenuated down growth of epithelium from a crypt (duct?). A female mother worm has entered a Brunner's gland at this point (figure 2).

Jejunum.—The invasion of the mucous membrane is similar to that noticed in the duodenum, though not as heavy; occasionally the submucous coat is invaded by a larva or a female mother worm.

It is seen, then, that there is definite injury done to the mucosa by the invading mother worm and by the presence of embryos, thus affording portals of entry for bacteria and other pathogenic micro-organisms. From this it was thought that possibly strongyloidosis made the host more susceptible to tuberculosis or other infections through the intestinal tract, but it does not appear that there is any etiological relationship between the several infections. The matter, however, is being carefully looked into.

METHODS OF CULTIVATION.

Nearly all the stools were "salt" stools. Several portions of these—three to five cubic centimeters—were placed in the center of Syracuse staining dishes and well moistened with sterile tap water, always leaving a margin of fluid surrounding the feces. Fluid feces required centrifuging and the sediment was used. Animal stools were treated in the same way, some of which had become quite hard and required teasing apart, yet they furnished positive cultures. The culture dishes were placed on a shelf near a window

where they got abundance of sunlight. Loss of fluid due to evaporation was made up by adding sterile tap water.

Solid media suggested by Dock and Gage did not seem to have any special value. They became overgrown with bacteria, etc., dried too easily, and did not transmit sufficient light. Larvæ and adults showed a tendency to crawl away to the margins where they would become dried out. Specimens could not be removed from the medium with any facility. The cultures in feces water kept in Syracuse glasses or in inverted Stender dishes seemed to resemble closely the natural condition of larvæ and sexually differentiated forms in moist soil, and these methods were adhered to throughout the experiments.

Sexually differentiated males and females prefer the deeper layers of the culture or the deeper portions of the margins. Rhabditiform larvæ could be seen scattered through the fluid, while filariform larvæ were seen crawling over the fecal mass or on the extreme outer edge of the margin of the fluid, apparently trying to escape, their movements being very restless and active and of a boring, penetrating character.

When it was desired to find strongyloides in lightly infected cases, cultures of the stools in Petrie dishes were used.

With the dissecting microscope, the larvæ can be seen after a few hours in the clear margin of fluid, and from time to time larvæ and sexually differentiated forms can be withdrawn with a pipette under the microscope. Rhabditiform or filariform larvæ and sexually differentiated forms can in this way be segregated or isolated. It was found convenient to isolate and segregate larvæ and adults in a small amount of feces water upon the under surface of a Stender dish cover, four and a half centimeters in diameter. This was placed bottom side up in the center of the under surface of a large Stender dish cover, seven centimeters in diameter. The larger Stender dish could then be vaselined and placed over the culture for protection and to prevent contamination and desiccation. Pure cultures of the direct and indirect modes were kept in this way for many days and studied not only under the dissecting microscope but with higher powers. By using Syracuse staining dishes, several cultures may be placed one on top of the other to

avoid drying, and larvæ kept in this way were alive and active for twenty-five days and were active when finally destroyed.

THE HETEROGAMY OF THIS GENUS OF NEMATODES.

Although a few scattered observations on parasitized natives of the tropics have been made, practically all the cultural work on strongyloides has been done in the temperate zone from individuals who acquired their infection there; consequently, the direct mode of development has been the one most frequently studied, for, as Leichtenstern[10] and others have shown, this is by far the commonest mode in the temperate zone. None of the writers on the subject has given a satisfactory explanation of the heterogamous mode of development of members of this genus of nematodes; *i. e.*, the presence of a free-living and a parasitic generation, the progeny of which are sometimes of the filariform, or direct generation, and sometimes of the rhabditiform, or indirect, sex-free generation.

Leichtenstern believed that the direct or indirect phase depended on the age of the mother worm—that the youthful mother worm produced the rhabditis generation, or indirect phase, while the older mother worm showed a tendency toward the direct metamorphosis. He and Grassi were also of the opinion that the direct phase was more dependent on temperature, moisture, and atmospheric conditions. The first of these hypotheses was nullified by Wilms, who fed to a man a culture of the direct phase, which developed into mother worms, yet instead of larvæ appearing in the indirect phase (youthful mother worm), as should have happened, according to Leichtenstern, both direct and indirect phases appeared.

The effect of moisture, temperature, and atmospheric conditions, as I shall show later, is not to alter the anlage, but merely to limit or inhibit the development of larvæ or the sex-free generation.

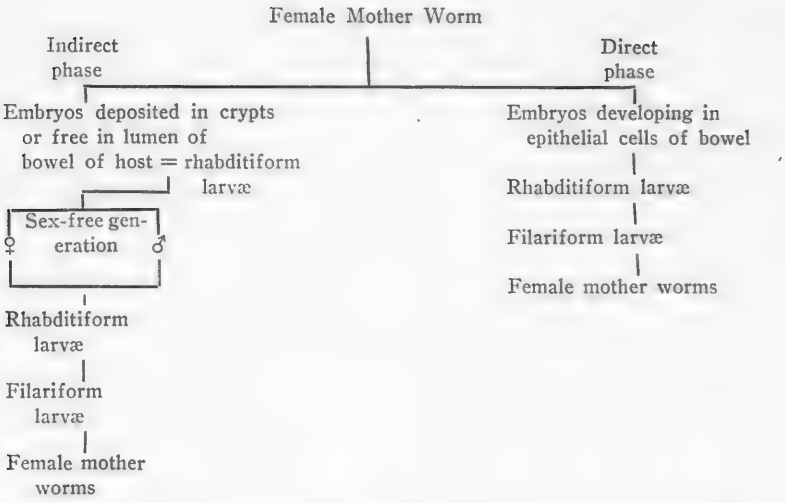
There is a marked preponderance of the direct mode of development in cultures obtained from natives of the temperate zones, and it will be shown that there is an equal preponderance of the indirect phase among natives of the tropics. The direct phase then may represent, as Leichtenstern believed, an adaptation of the worm to a colder climate. Now, while it may be true that as infections have been conveyed from warmer to colder regions, the facility

[10] Leichtenstern, *Deutsch. med. Wchnschr.*, 1898, xxiv, 118.

with which the direct strain can infect in colder climates has resulted in their preponderance there, it still remains to be shown by further experiments whether the direct mode larvæ have a tendency toward reproducing more or less exclusively their own kind, because if they do not, the preponderance of the direct phase could hardly arise in this way. Wilms's experiment indicates that the direct mode larvæ have no tendency toward reproducing exclusively or preponderatingly their own kind. On the other hand, considering the number of instances of the direct mode of development among natives of the tropics, Dr. Stiles's suggestion that the direct metamorphosis is an approach to more perfect parasitism, may be the true reason for the origin of this phase.

Rationale of the Mode of Origin of the Direct and Indirect Phase Larvæ.—It must be kept in mind that the female mother worm burrows into the mucosa more or less deeply, occasionally into the submucosa. Ova are found in the epithelial cells and in the female mother worm. Larvæ are developed undoubtedly in two locations: (*a*) the epithelium of the mucosa; and (*b*) those extruded from the mother worm into the crypts and lumen of the bowel. The embryos maturing in the tissues of the mucosa and escaping as rhabditiform larvæ are certainly placed under special circumstances with regard to nutrition, and this suggests the possibility that larvæ from the two sources may have different functions. The parasitic intra-epithelial cell-grown larvæ developing into one phase, and the larvæ extruded as ova directly into the bowel or crypt from the female mother worm developing into the opposite phase—direct or indirect. From a comparative study of my material, I am inclined to believe that the parasitic cell-grown larvæ develop into filariform larvæ, always of the direct phase, while larvæ extruded into the crypts or the lumen of the bowel from the mother worm develop always into the sexually differentiated or indirect phase. This explanation fits in with a number of observations made on the relative proportion of larvæ and mother worms in the tissue compared with direct and indirect phase larvæ in cultures of man and animals.

The life history of S. stercoralis and of the strongyloides found in the monkey and coati might be expressed schematically thus:—



The Preponderance of Direct or Indirect Phase Larvæ in Different Individuals.—Cultures derived from both Spanish and West Indian laborers were studied to ascertain the degree of preponderance of the direct mode of development (temperate zone type) among the former class; for considering the improved sanitary conditions in which they lived, most if not all of this class must have acquired their infections in Spain.

In table II is seen the striking difference between the character of infections in the Spanish and the natives of the tropics. The Spanish always show the direct phase, while only about 50 per cent. of the natives of the tropics show it. On the other hand, nearly 50 per cent. of the natives of the tropics show a pure culture of the indirect phase (type of the tropics). In none of the Spanish cases, all presumably infected in Spain, was there one of the purely indirect mode; although in Lopez there were extremely few direct phase larvæ, and in Bal they were about equally balanced. The Spanish, as a rule, were more lightly infected than the blacks, and it is more difficult to detect the indirect phase when they are present in small numbers, because the sexually differentiated adults have a tendency to remain in the deeper, opaque portions of the culture.

Attention is called to the cultures from Carrington, Hall, and Husband, purely tropical strains in negroes, yet showing a preponderance of the direct mode of development.

TABLE II.

Name.	Nativity.	Length of time in tropics.	Color.	Mode of development.
Quadrufia	Colombia	Life	White	Direct and indirect phase.
Stamford	Barbados	Life	White	Indirect phase.
Fitzsimmons	Barbados	Life	Black	Indirect — no direct phase.
Simmons	Barbados	Life	Black	Indirect — no direct phase.
Carrington	Jamaica	Life	Black	Direct and indirect phase.
Husband	Jamaica	Life	Black	Direct phase.
Hall	Jamaica	Life	Black	Direct and indirect phase.
Kelly	Monserrat	Life	Black	Indirect and direct phase (chiefly the indirect phase).
Small	West Indies	Life	Black	Direct phase.
Morgan	Monserrat	Life	Black	Indirect phase.
Downs		Life	Black	Indirect phase.
Elgaraldad	Spain	3½ years	White	Direct and indirect phase.
Arroyo	Spain	29 months	White	Direct phase.
Asual	Spain	27 months	White	Direct phase.
Sanchez	Spain	13 months	White	Direct and indirect phase.
Lopez	Spain	40 months	White	Direct and indirect phase (chiefly the indirect phase).
Armcibaz	Spain	38 months	White	Direct and indirect phase.
de la Cruz	Spain	30 months	White	Direct phase.
Garcia	Spain	26 months	White	Direct and indirect phase.
Gomez	Spain	28 months	White	Direct phase.
Seranio	Spain	36 months	White	Direct phase.
Bal	Spain	18 months	White	Indirect and direct phase.
Lopez	Spain	36 months	White	Indirect and direct phase.

The Proportion of Individuals of the Direct and Indirect Generations.—The relative proportion of filariform larvæ of the direct phase to the males and females of the indirect phase varies decidedly in different cases and from time to time in the same individual, as is shown by the following notes from several cultures:—

Hall, West Indian negress. In this patient there were many larvæ of the direct phase and fewer sexually differentiated adults, the males of the latter generally predominating. In one culture there were:—

Filariform larvæ, direct phase, 31.
Sexually differentiated males, 3.
Sexually differentiated females, 1.

Elgaraldad, Spanish:

Filariform larvæ, direct phase, 25.
Sexually differentiated females, 2.
Sexually differentiated males, 1.

Kelly, West Indian negro:
Filariform larvæ, direct phase, 1.
Sexually differentiated forms, 20 to 30.
Fitzsimmons, West Indian negro:
Filariform larvæ, direct phase, none.
Sexually differentiated females, many.
Sexually differentiated males, none detected.
From another culture, nine days later, there were:
Filariform larvæ, direct phase, none.
Sexually differentiated males, 1.
Sexually differentiated females, 5.
Many of the females died without fecundation.
Simmons, West Indian negro:
Filariform larvæ, direct phase, none.
Sexually differentiated males and females, an equal number.
Carrington, West Indian negro:
Filariform larvæ, direct phase, 8 to 10.
Sexually differentiated males, 1.
Sexually differentiated females, 1.
Lopez, Spanish:
Filariform larvæ, direct phase, 1.
Sexually differentiated males, 1.
Sexually differentiated females, 12.
Bal, Spanish:
Filariform larvæ, direct phase, 7.
Sexually differentiated males, none.
Sexually differentiated females, 4.
Lopez, Spanish:
Filariform larvæ, direct phase, 1.
Sexually differentiated males, 3.
Sexually differentiated females, 26.
Quadrufia, Colombian:
Filariform larvæ, direct phase, 8.
Sexually differentiated adults, 3.

EXTERNAL FACTORS THAT INFLUENCE VIABILITY OR GROWTH.

A number of experiments were carried out for the purpose of determining the viability of the worms when exposed to sunlight, desiccation, cold, and larvicides.

The Effect of Sunlight.—In some of the culture dishes, the filariform larvæ swarmed in locations favorable for warmth or shade. At times they were on the side nearest the morning sun and at other times on the opposite side in the shade. This was the only effect observed. The larvæ could also be segregated by means of the electric light.

The Effect of Desiccation.—Rhabditiform larvæ and sexually differentiated adults are more sensitive to the effects of desiccation than are filariform larvæ. The latter undoubtedly have more impenetrable sheaths. Filariform larvæ, when exposed in thin films at a temperature of 82° F., can not be revived if the film has become quite dry; but if there is the slightest trace of visible moisture, they revive upon the addition of water, although they may have been motionless for an hour. As the films evaporate the larvæ become segregated in the center where there is moisture.

The Effect of Cold.—There is no tendency, in a purely tropical strain, at least, when subjected to the effect of a lowered temperature, for the direct mode to predominate in a culture over the indirect mode, and this indicates that the determination of the metamorphosis of S. stercoralis and allied forms by the direct or indirect mode depends on factors not altered by changes in temperature. Larvæ of the indirect phase can be obtained in cultures at temperatures reached in the temperate zones during the summer time (68° to 72° F.). My experiments show that the retarding effect of cold limits the egg production and the ability of these ova to develop into larvæ of the indirect phase. The lowered temperature prevents the growth of the protozoa, bacteria, and yeasts usually seen in successful cultures kept at room temperature, and thus inhibits the growth of the larvæ by limiting the food supply. Development is merely arrested because the sexually differentiated generation is more sensitive to outside influences. The larvæ which should develop into filariform larvæ of the direct phase do so. The cold does not effect the anlage.

Several cultures from Kelly and Lopez, which were nearly pure indirect phase larvæ, were exposed to varying degrees of cold in diffused daylight in a large refrigerator, the temperature of which remained constant and was recorded by means of a self-registering thermometer.

Cultures exposed to a temperature of 67° to 74° F. developed as rapidly as those kept at room temperature (77° to 84° F.). Cultures kept at a temperature of 44° F. for 24 hours or more failed completely to develop, and the larvæ were all dead after two or three days. Cultures at 58° F. failed to develop and most of the

larvæ died within three days, although if alive when they were removed to room temperature (77° to 84° F.), or to a temperature of 68° to 72° F., they developed into normal sexually differentiated adults and produced ova and larvæ.

When cultures of larvæ were kept at a temperature of 57° to 68° F., their development was delayed greatly, so that sex-ripe adults were not seen until the sixth day, whereas at room temperature they would have been seen at the end of 24 hours; and, although the cultures were kept for six days longer, it was observed that the females never contained more than six or eight eggs instead of the usual thirty or more, and that none of these ever developed into living larvæ.

The Effect of Larvicidal Agents.—This must be considered with regard to (*a*) the destruction of larvæ in dejecta, and (*b*) the destruction of the female mother worm and of larvæ in the intestinal lumen.

The sensitivenesss of rhabditiform larvæ and of sexually differentiated worms to desiccation has been referred to above. They are always actively sucking fluid and food particles into their esophagus so that chemical substances in solution or in a finely divided state, such as emulsions, are rapidly effective as larvicides. Filariform larvæ are not so sensitive, but they ultimately succumb to the larvicides fatal to the rhabditiform larvæ, if the contact is long enough for their chitinous envelope to be penetrated. They are so active, however, that they might wriggle out of the larvicide before it could penetrate and destroy them.

The effects of larvicidal agents on the female mother worm were not tested, but the value of thymol as a destructive agent for filariform larvæ was determined, a slightly alkaline solution of thymol in four dilutions being used.

1:1000	1:2000	1:8000	1:1600
Filariform larvæ died in a few minutes.	Some filariform larvæ died in 50 minutes.	One half-grown sexually differentiated male living at the end of 24 hours. Filariform larvæ dead.	Filariform larvæ living at the end of 22 hours.

The resistant as well as the infecting form is the filariform larva, and where it is desired to disinfect feces chemically, instead of disposing of them by the usual sanitary methods, efforts must be directed against the more resistant and active filariform larvæ.

The following substances were tested:—

Phenol	Cupric sulphate	Sulphuric acid
Larvicide	Ferrous sulphate	Sodium hydroxid
(An alkaline cresol soap)	Arsenious oxid	

Large numbers of filariform larvæ were placed with a volume of water in a Stender dish and an equivalent volume of the chemical in solution was added to them. The final dilution of the larvicidal agent was 0.5 per cent. Two hundred to three hundred larvæ were exposed in each instance with very little organic matter. The death of the larvæ was determined with the microscope.

The larvicide (an alkaline cresol soap) killed all the larvæ in thirty-one minutes.

The alkaline solution of arsenious oxid was destructive in fifty-five minutes.

The solutions of sulphuric acid, phenol, cupric sulphate, and ferrous sulphate did not kill in one hour, but all the larvæ were dead at the end of twenty-four hours.

THE STRONGYLOIDES OF MONKEYS AND ANT BEARS.

These were studied for the purpose of ascertaining if they might be a cause of diarrhea or anemia in their hosts and to throw some light on the mechanism of the heterogamy of the genus. These nematodes, while resembling S. stercoralis and S. fülleborni, are undoubtedly distinct species, and their study emphasizes the fact that while strongyloides worms invade the intestinal wall and cause some low grade inflammatory change, and possibly anemia, as in hookworm disease, they do not cause diarrhea.

The Strongyloides Found in the Monkey.—Cebus hypoleucus is the commonest species of monkey in this locality. Four of six especially examined have yielded strongyloides larvæ in cultures. Two of the individuals at autopsy contained in the upper portion of the small bowel many female mother worms, ova, and larvæ. Cultures from one of these were carefully studied. The sexually differentiated indirect phase predominated, there being about five

adult individuals of the indirect phase to one filariform larva of the direct phase. In one culture there was a very marked preponderance of males over females, thirty males to one female; while in another culture the females preponderated in the proportion of twelve to one male.

An examination of sections shows that there are relatively few embryos imbedded in the intestinal mucosa compared with the number of attached female mother worms. This apparently is in confirmation of my view that the direct generation is derived from embryos deposited in the epithelial cells of the mucosa, there being in cultures from this monkey a small number of filariform larvæ of the direct phase corresponding with the smaller number of embryos found in the epithelial cells.

Description of the Strongyloides. The female mother worm (figure 8) was found at autopsy and in sections in large numbers in the duodenum. The worm has the usual elongated shape, an attenuated anterior end and a long esophagus. Its length was 3.200 mm., and its breadth 0.045 mm. The proportion of breadth to length is 1:66.

The skin is smooth and the simple mouth is surrounded by six very small papillæ.

The tail end is pointed and slightly recurved.

The vulva is placed behind the middle of the body, 1.920 mm. posterior to the mouth.

The esophagus is elongated without a bulb or chitinous teeth. Its length is 0.768 mm., or about one fourth the length of the worm.

The uterus in several worms contains 6 or 8 ova in various stages of segmentation, but none contained motile embryos. Smears from the mucosa contained many strands of ova, 8 to 10 in number, evidently expelled from mother worms. The ova measured 0.064 by 0.040 mm.

The newly hatched larvæ in scrapings from the mucosa measure in length 0.240, in width 0.016 to 0.020 mm. They increase rapidly in size and soon present the characteristic wriggling motion of rhabditiform larvæ—not the penetrating motion of filariform larvæ. From mouth to base of the esophagus the distance is 0.080 mm., one third the length of the larvæ. After twenty-four hours in cultures, the rhabditiform larvæ measure in length 0.560 mm., in width 0.024 mm.

In cultures, most of the larvæ develop into the indirect phase; very few (about one fifth in one culture) into the direct phase. Filariform larvæ of the direct phase were seen during the second day, and filariform larvæ of the indirect phase not until the third day.

Filariform larvæ of the direct phase measure in length 0.560 mm., in width 0.022 mm.

Filariform larvæ of the indirect phase when forty-eight hours old measure in length 0.704 mm., in width 0.021 mm.

The indirect phase appears as sexually differentiated males and females in forty-eight hours. They resemble very closely the sexually differentiated race of S. stercoralis, but are somewhat smaller. The vulva of the female is not nearly so prominent as the one figured by von Linstow for S. fülleborni, and there is no diminution of the caliber of the female just posterior to the vulva.

The male is 0.632 to 0.800 mm. in length, and 0.032 to 0.044 mm. in width, increasing slightly in size after twenty-four hours.

The distance from the mouth to the base of the esophagus is 0.136 mm., and from the anus to the tip of the tail, 0.064 mm. Each male has two spicules, 0.035 mm. to 0.036 mm. long, resembling in shape a short cow's horn. The rhomboid chitinous basal plate in relation with the chitinous spicules is 0.024 by 0.012 mm. in area. The male has a pre-anal and a post-anal papilla, the latter being smaller and not so prominent as the former.

The female is 0.872 mm. in length and 0.048 mm. in width. The distance from the mouth to the base of the esophagus is 0.168 mm. From the mouth to the vulva the distance is 0.416 mm. From the mouth to the anus, 0.764 mm.; and from the anus to the posterior tip, 0.108 mm. Ova are 0.034 to 0.056 mm. and 0.040 to 0.068 mm.

One experiment was carried out in which a four day old fecal culture containing many filariform larvæ was fed by mouth to another monkey (*Cebus*), cultures from the stools of which had been free from larvæ on several examinations. After the feeding, no larva was seen within six days, but on the 29th and 43d days after feeding, ova containing moving embryos were found in the stools.

This corresponds with Van Durme's statement that he never found larvæ in the fresh stools of chimpanzees, but always ova.

This is significant, too, in connection with my view that strongyloides larvæ of the indirect mode are derived from ova extruded from the mother worm directly. For ova could hardly escape from epithelium cells of themselves, while larvæ easily do so.

Histopathology of the Intestinal Mucosa. Ileum.—The epithelium of the villi here and there contains imbedded ova, but the deeper layers of the mucosa in the crypts show no invasion and none is seen in the submucosa. A number of cross sections of adult worms are seen (figures 5 and 6). Some of them are in the lumen of the bowel, while others lie just beneath the epithelium of a villus. The mucosa is rich in lymphoid and plasma cells and contains many eosinophilic leucocytes.

The Strongyloides of the Ant Bear, Nasua Nasica Panamensis.—Both coati were immature specimens, male and female. The male had a mild, simple infection, the female mother worms at autopsy being located in the posterior three inches of the colon. The female

coati was heavily infected, the female mother worms being found in the first four inches of the bowel below the pylorus. There were also many small strongyles and several cestodes in the middle portion of the bowel, while in the posterior three inches of the colon there were about a dozen attached specimens of echinorhynchus.

The strongyloides worm resembles others of this genus very closely. The vulva of the female of the sexually differentiated generation, however, has very prominent projecting lips, and one specimen had a depression posterior to the vulva, as in S. fülleborni, but this was not a constant feature.

A pure culture from the male coati was studied, and the indirect mode of development seemed to preponderate greatly. There were always, however, a few larvæ of the direct phase. One culture contained the proportion of one filariform larva of the direct phase to two males and five females of the indirect phase.

The female mother worms collected at autopsy and placed in diluted saline solution with chyme were actively motile after twenty-four hours. This female has the appearance of the female mother worms typical of this genus, with a long attenuated body and anterior end. The lips of the vulva protrude slightly and the worm contains two, three, or four ova, most of which are unsegmented; a few have divided into two or four segments, but none contained motile embryos.

Length of female mother worms	2.080–2.400 mm.
Breadth of female mother worms	0.052–0.048 mm.
Mouth to base of esophagus	0.880 mm.
Mouth to vulva	1.744–1.472 mm.
Vulva to posterior tip	0.656–0.608 mm.
Anus to posterior tip	0.060–0.072 mm.
Ovum	0.056 by 0.032 mm.

There is a small posterior anal papilla just posterior to the anus, and the tip of the tail is slightly bulbous.

One immature worm measuring 1.840 mm., and one measuring 1.712 mm. were seen. Neither of these immature worms contained ova.

Cultures of feces five hours old contained rhabditiform larvæ, in length 0.320 mm., in width 0.016 mm. The distance from the mouth to the base of the esophagus was 0.092 mm. The mouth of the larva has six small papillæ, four larger and two smaller. The vestibulum oris is 0.004 mm. long.

The sexual node is elongated and placed in about the middle of the body. Its length is 0.040 mm. and its width is 0.088 mm. At the end of twenty-four hours the rhabditiform larvæ are 0.400 mm. long and 0.024 mm. wide. From the mouth to the base of the esophagus is 0.112 mm.

The males and females of the indirect phase are fully developed at the end of forty-eight hours.

Length of male	0.800–0.928 mm.
Breadth of male	0.048–0.040 mm.
Mouth to base of esophagus	0.136–0.120 mm.
Anus to tip of tail	0.064 mm.

The male has two curved horn-shaped spicules, 0.036 mm. in length and a rhomboid basal plate attached near the exit of the spicules 0.026 by 0.009 mm.

The male has a small pre-anal papilla and a small post-anal one. The anterior one is separated from the exit of the spicules by a distance equal to the length of a spicule (0.036 mm.).

The female of the indirect phase has a vulva with protruding lips.

Length of female	1.328 mm.
Breadth of female	0.096 mm.
Mouth to base of esophagus	0.192 mm.
Mouth to vulva	0.672 mm.
Anus to posterior tip	0.088 mm.

The posterior end is acutely pointed.

As seen in cultures, the female contains a variable number of ova, some with thirty or more and others with eight or ten embryos, some of them being motile. The embryos are seen escaping alive from the mother.

The length of rhabditiform larvæ of the indirect phase is 0.256; the width, 0.016 mm.

In one culture made on July 16, there were numerous males and females on July 21, and two days later there were so many larvæ present that it suggested the possibility of the continued production of embryos from the sexually differentiated generation. This was not confirmed, however.

Histopathology of Tissue from the Coati. Coati No. 298.—Several adult mother worms are seen penetrating the mucosa in various locations—surface epithelium and deeper in the crypts (figure 4). A few single scattered ova are imbedded between the epithelial cells of the crypts and several of the crypts contained rhabditiform larvæ. The imbedded ova usually are segmented or contain embryos. The interglandular stroma is rich in round cells and those of the fibroblastic type, and there is also a diffuse infiltration of the stroma by polymorphonuclear leucocytes; but there are no areas of necrosis.

Coati No. 296.—At autopsy several female mother worms were found and removed. In the sections of adjoining tissue, however, none are seen, but there are a few imbedded ova and rarely a larva. The larvæ were seen in greatly dilated cystic crypts associated with desquamated epithelium and leucocytic plugs. There is some increase in the number of cells in the interglandular stroma of the round plasma cell and fibroblastic type. There is also an increased number of eosinophilic leucocytes and a slight but definite exudation of polymorphonuclear leucocytes. The cells in the interglandular stroma show a good many mitotic figures.

Considering the great number of larvæ found in the cultures of feces on some occasions from this animal, there are very few imbedded ova in the section, and this indicates that most of the larvæ are derived from the mother worm as she expells them free into the crypts or the lumen of the bowel.

SUMMARY.

From a study of strongyloides infections in man and animals in this region, it has been determined, in confirmation of the view of Grassi, Calmette, and others, that they are not causative factors in the production of diarrhea. The mother worm, however, burrows into the mucosa and deposits her ova there. Certain tissue reactions take place and are evidenced by the cellular proliferation in those portions of the intestines occupied by the nematodes.

In animals, there is an associated anemia, not positively attributable to the strongyloides, but, on the other hand, not attributable to any other cause. It is possible that S. stercoralis may cause some degree of anemia in man. The amount is indeterminable in this region among hospital cases on account of the associated hookworm disease or malaria.

Portals of entry for various microörganisms are made by the female mother worm and her larvæ in the small intestine, and, while no case of general bacterial infection has been proved to have arisen in this way, its occurrence is possible and highly probable.

In the cultures of strongyloides of man, there is among natives, who presumably are infected with purely tropical strains, a very marked predominance of development by the indirect or sexually differentiated mode; in some cases, an absolutely pure culture of the indirect mode larvæ being obtained. There are, however, natives, cultures from whose stools contain from a single filariform larva of the direct phase up to a very definite predominance of this mode. Cultures from natives of the temperate zones contain a marked predominance of the direct phase larvæ.

The presence of the filariform (direct mode) larva is perhaps best accounted for by its being an attempt at more perfect parasitism (Stiles).

From a correlation of culture study with the results of a histological examination of the invaded mucosa, this explanation of the derivation of the two phases is suggested. The mother worm in the intestinal tract has two kinds of progeny: (*a*) those expelled into the crypts or lumen, and (*b*) those imbedded in the intestinal wall. One lot becomes larvæ of either the direct or indirect phase;

the other lot of the opposite phase. It appears to me that the intra-epithelial cell-developed larvæ furnish the direct phase, while the embryos expelled directly from the mother become the indirect phase larvæ.

This will require for its confirmation a study of cultures in connection with a histological examination of the infected intestinal wall.

Cold merely inhibits the development of the larvæ into either the filariform (direct) or into the sexually differentiated adults, and does not alter the anlage.

The resistant as well as the infecting form is the filariform larva, and all chemical larvicides must be directed against this form. Thymol and an alkaline cresol resin soap were found to be effective larvicides for the filariform larvæ.

Two new strongyloides of the monkey and ant bear are described. Cultures of these nematodes show a predominance of the indirect mode of development.

I wish to express my thanks to the various members of the hospital and asylum staff for courtesies, and to the Chief Sanitary Officer for permission to publish this paper.

EXPLANATION OF PLATES.

Plate 1.

Fig. 1. S. stercoralis. Ova and larvæ imbedded in the epithelium of the crypts; cross and oblique sections of mother worms in the interglandular stroma.

Fig. 2. S. stercoralis. Mother worm in Brunner's gland.

Fig. 3. S. stercoralis. Embryos and some free larvæ in the bottom of a crypt.

Plate 2.

Fig. 4. S. nasua. Mother worm and ovum imbedded in mucosa.

Fig. 5. S. cebus. Mother worms.

Fig. 6. S. cebus. Mother worms.

Plate 3.

Fig. 7. S. cebus. Filariform larvæ.

Fig. 8. S. cebus. Mother worm.

Fig. 9. S. stercoralis. Female of the free-living generation.

Fig. 10. S. stercoralis. Two males of the free-living generation.

FIG. 1.

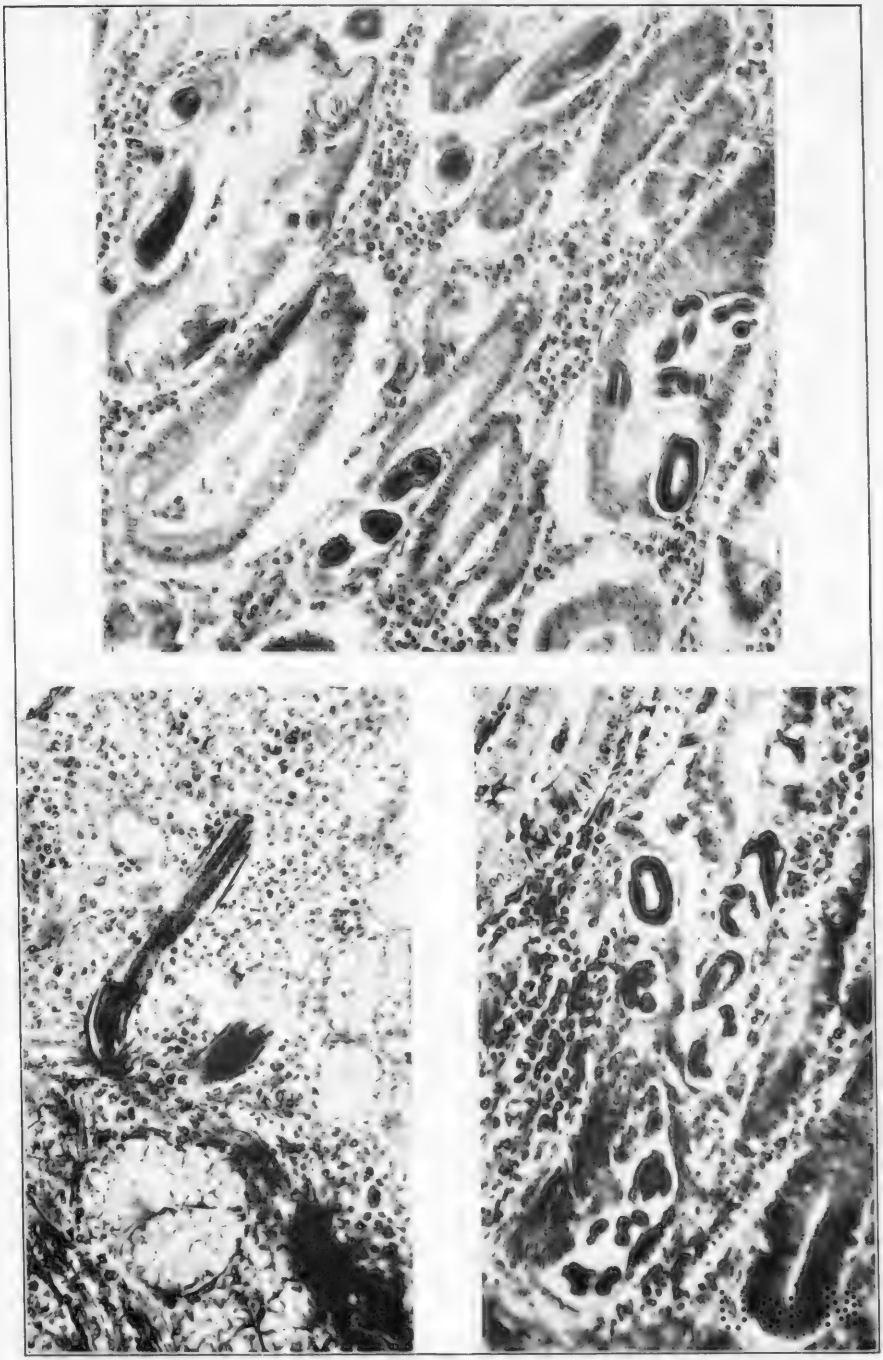


FIG. 2.

FIG. 3.

Mau

FIG. 4

FIG. 5.

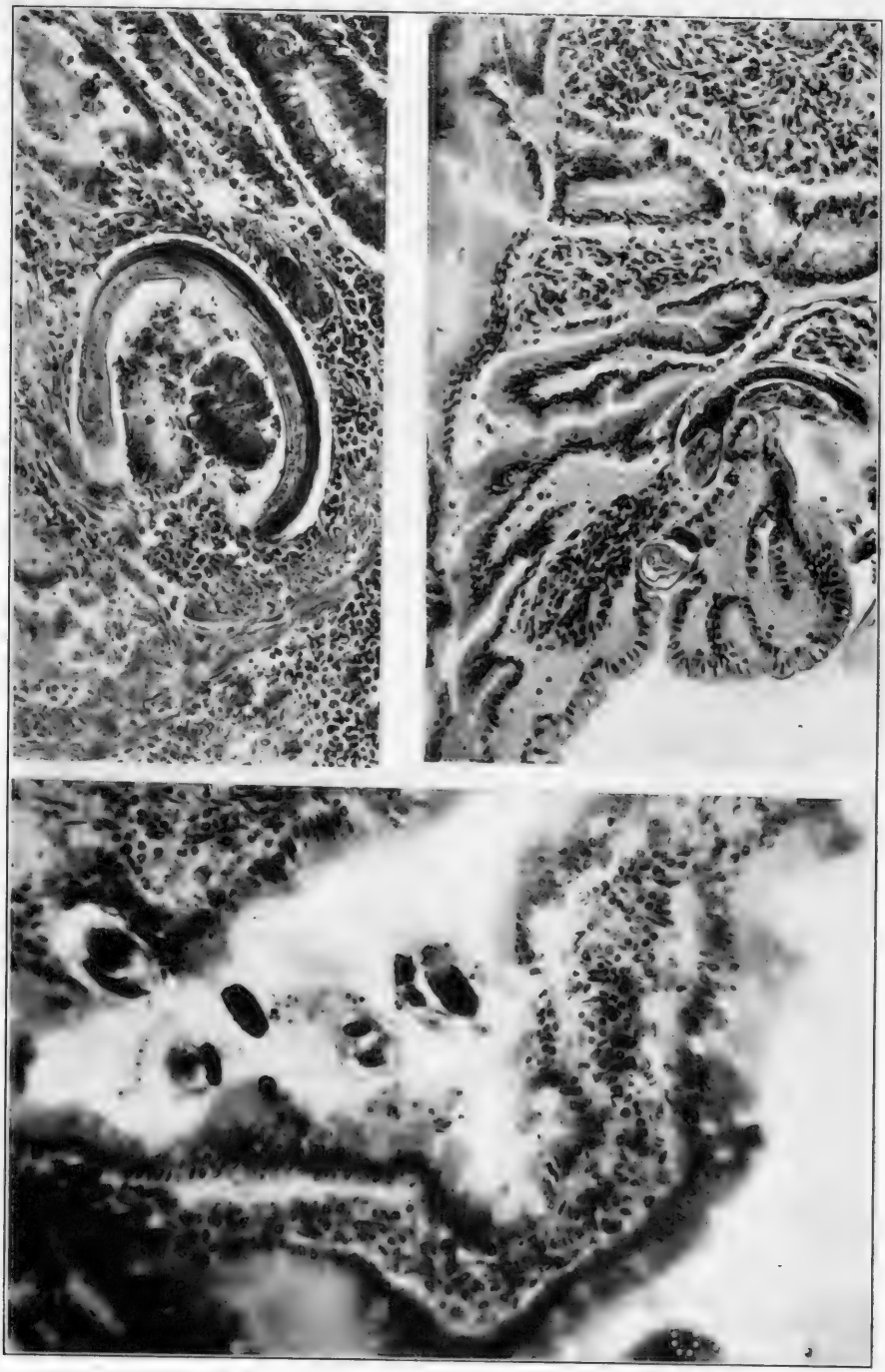


FIG. 6.

Mau

FIG. 7. FIG. 8.

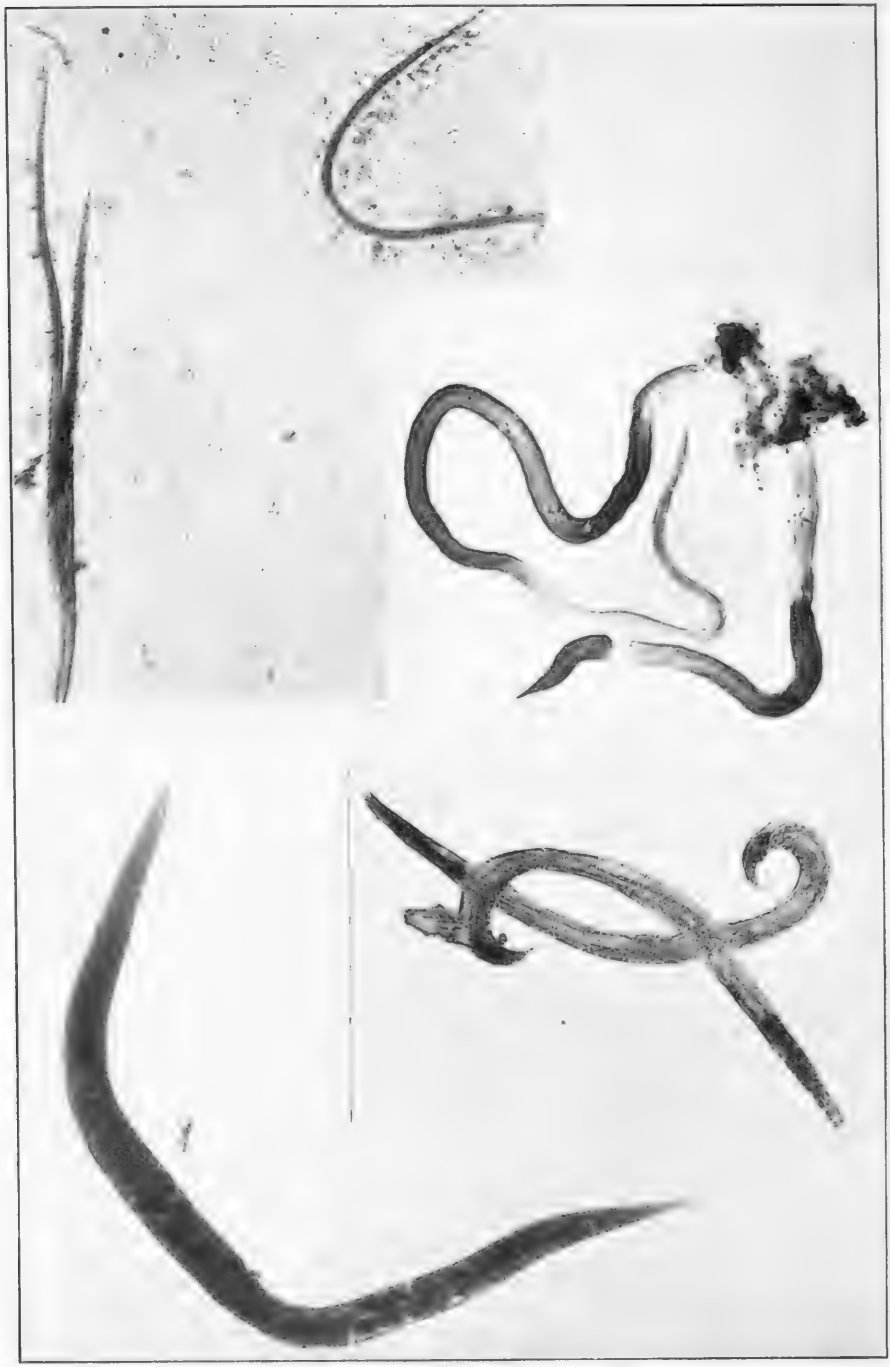


FIG. 9 FIG. 10.

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ON THE TOXIC ACTION OF CERTAIN NORMAL SERA AND ITS RELATION TO ANAPHYLAXIS.*

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INTRODUCTION.

Although well-known for many years, the inherent toxic action of certain normal blood sera when injected into animals of different species, has recently attracted renewed attention owing to the many points of similarity in the symptoms of animals dying of such injections and of those dying of anaphylactic shock. The older studies of phenomena belonging in this category followed closely in the footsteps of experiments on transfusion, and as early as 1666 a commission of the London Royal Philosophical Society reported deaths following transfusion, alleging intravascular coagulation as the probable cause of death.

The cause of death following injections of foreign whole blood, blood cells, and serum has, since that time, occupied the attention of many workers whose studies need not be reviewed for the purposes of this paper. Chief among them were Magnani, Brown-Sequard, Magendie, and, more recently, Naunyn, Landois, and Ponfick.[1]

The work of Landois is of special interest to us in the present connection, in that he worked with blood serum free from cells, and attempted to bring into relation the occurrences after the injection of animals with the action of the serum upon the cellular blood elements *in vitro*. Landois observed both the solution of hemoglobin and hemagglutination, and was led to regard the action of serum upon erythrocytes as the primary cause of death after transfusion. His conception of the mechanism is apparently twofold. On the one hand, he believed that when small quantities of blood were transfused, a formation of fibrin (stroma-fibrin) was initiated in the stroma of the injured erythrocytes which led to coagulation and thrombosis in the capillaries of the central

* Received for publication, May 1, 1911.

[1] A brief historic review of this work can be found in the paper of Coca (1), *Virchows Arch. f. path. Anat.*, 1909, cxcvi, 92.

nervous system and lungs. In the case of the transfusion of rabbits' blood into dogs, he attributed death to embolism in the pulmonary vessels due to "Massenhafte Verklebung der Kaninchenzellen im Hundeblut"—or, in other words, to hemagglutination.

Ponfick and others have disputed the validity of Landois' conclusions, but the basic principles of his explanations have been upheld within recent years by workers who have gone over the same ground with the aid of more modern methods. Two careful researches have appeared during the last two years in which the problem has been approached by different routes, but in which the general conclusions show much agreement. Coca (1), investigating the cause of death following the injection of washed blood cells into animals of different species, concludes that, in these cases, death is due to mechanical obstruction of the pulmonary circulation owing to agglutination of the injected cells. It is important to note, however, that he adds in his conclusions the following paragraph: "The mere presence of specific agglutinins does not suffice, in the injection of 'toxic' erythrocytes, to occlude the pulmonary circulation. The coöperation of another factor must be assumed—a factor probably found in the capillary walls."

Loeb, Strickler, and Tuttle (2) investigated the cause of death following the injection of normal dog and beef sera into rabbits. They correlated their animal experiments carefully with the action of the sera *in vitro* upon the blood elements of rabbits, and utilized the property of hirudin to inhibit the coagulation of blood, finding, in the case of dog serum, that injections of hirudin, while not always preventing death, at any rate prolonged life or necessitated an increase in the lethal dose. The conclusions of these authors are as follows: "Death following the injection of foreign serum is brought about by obstruction of the pulmonary circulation either by heaps of agglutinated erythrocytes or by fibrinous plugs. Dog serum and beef serum represent two different types. In the case of dog serum, hemolysis of the blood cells of the recipient liberates substances attached to the stromata, which hasten coagulation. In consequence, fibrin is formed which is carried into the pulmonary vessels and occludes them. In the case of beef serum, death is due to hemagglutination."

It is seen that, in the researches above quoted, the death of animals induced both by repeated injections of red cells and that caused by the single injection of heterologous sera is attributed to the induced coagulation of blood or to embolism due to hemagglutination.

Direct association of the toxicity of hemolytic immune sera upon animals with hemolysis was, of course, a thought suggested obviously by the early work of Belfanti and Carbone. Detailed analysis of this was carried out, in 1905, by Battelli (3) and more recently by Friedemann (4), both of whom see definite reason for correlating this phenomenon with anaphylaxis.

Battelli, working with dog and guinea pig erythrocytes and with rabbits immunized with these cells, comes to the following conclusions:—

1. Extracts of the erythrocytes of dogs and guinea pigs (obtained by laking with distilled water) produce fall of blood pressure and are toxic when injected into the veins of a rabbit immunized with these cells.

2. Such an extract has similar toxic effects upon a normal animal when subjected before injection to the action of the serum of an immunized rabbit.

3. The serum of an immunized rabbit has gained properties which permit its transforming inactive substances of dog and guinea pig corpuscles into toxic substances.

The results of Friedemann are similar in significance. This author, working on anaphylaxis to erythrocytes in rabbits, found that animals which had been intravenously injected with beef blood would react with slight symptoms when reinjection was practised after one week, and often died when, after another week, a third injection was given. He found that the toxic action of the injected red blood cells was in direct relation to the hemolytic powers of the serum of the recipient rabbit. When he allowed specific amboceptor and complement to act upon corpuscles *in vitro,* toxic substances were formed even before hemolysis had been more than just initiated, when the supernatant liquid was still hardly reddened.

Friedemann's results have been corroborated by Uhlenhuth and Haendel (5) and, more recently, by Doerr and Moldovan (6).

Both Battelli's work and that of Friedemann dealt with the study of blood cells injected into immunized animals or of the reaction of such cells with immune hemolytic sera.

The toxic action of certain normal sera for animals of different species has recently been discussed by various writers (Doerr (7) and others) in connection with the work of Friedemann, and the opinion has been expressed that, in principle, both phenomena are closely related, and that both may probably be accepted as belonging to the category of anaphylactic reactions.

An analysis of the toxic action of certain normal sera from this point of view has been made by Uhlenhuth and Haendel, who, in studying the necrotizing action of beef serum injected into guinea pigs, attribute this action of the serum to a "complex process depending upon the coöperation of complement," but not identical with the hemolytic mechanism. The toxic action of such serum, however, they separate from the necrotizing action, concluding that this is independent of complement, and more thermostabile than either the mechanism causing necrosis or that responsible for hemolysis.

A recent publication by Doerr and Moldovan corroborates the results of Uhlenhuth and Haendel and, by further experiments, attempts to identify the phenomena observed with anaphylaxis. This work will be referred to in another place.

I.

The observations which follow are the result of an attempted analysis of the toxic action of normal goat serum upon rabbits.

The first experiments cited furnish an estimate of the degree of toxicity of the goat serum.

EXPERIMENTS DEMONSTRATING THE TOXIC ACTION OF UNHEATED NORMAL GOAT SERUM UPON RABBITS.

A few only of these experiments are given, since they merely demonstrate a well-known fact.

Goat I.—Adult female. The serum of this goat actively hemolyzed and agglutinated washed rabbit corpuscles. 1 c.c. caused almost complete hemolysis of 1 c.c. of a 10 per cent. rabbit corpuscle emulsion in salt solution.

Rabbit 1.—Weight 885 grams. 5 c.c. of fresh goat serum injected intravenously. Respiratory difficulty in 1 minute. Paralysis of hind legs; then general convulsions. Death in 2 minutes.

Rabbit 2.—Weight 885 grams. 4 c.c. of goat serum injected intravenously. Respiratory difficulty in 3 minutes. Micturition tinged with hemoglobin in 4 minutes. General convulsions and death in 4½ minutes.

Rabbit 3.—Weight 895 grams. 3 c.c. of goat serum injected intravenously. Respiratory difficulty in 4 minutes. Micturition not bloody. Weakness of limbs. Flattened out on floor in 5 minutes. Head retracted in 5½ minutes. Gasping respirations, lay motionless, with occasional convulsive twitching of legs. Died in 14 minutes.

Rabbit 4.—Weight 1,000 grams. 2 c.c. of goat serum injected intravenously. Micturition in 2 minutes. Gradually grew weak and sank to the floor. Flat on belly in 8 minutes. Convulsions in 54 minutes.

Experiments with similar results were carried out with the sera of two other goats—one an adult male, the other a female about two months old. The results in all cases showed definite toxicity, but less marked in the young goat than in the two adults. The symptoms following intravenous injection of the goat serum were so uniform that they may be summarized as follows:—

There was always a definite period of complete well-being after injection. This varied from one minute, when large doses were given, to five to ten minutes when the quantities given approached the minimal lethal dose.

In the case of larger doses—five to six cubic centimeters—there first appeared great respiratory distress, the respirations being at first rapid and shallow, then becoming slow and shallow with, finally, violent efforts at inspiration accompanied by gasping and spasmodic opening of the jaws. This was rapidly accompanied by paralysis, usually beginning in the hind legs. The animals fell upon the side and general convulsions preceded death, which occurred in from three to eight minutes.

When the doses were smaller, the onset was delayed but again began with respiratory difficulty. This was often followed by micturition which, in some cases, was hemoglobinuric. The animals then gradually sank to the ground, lying upon the abdomen with the limbs stretched out to the sides, apparently too weak to rise. At this stage, the only symptom of motor irritation was occasional intermittent retraction of the head, and the animals would often remain in this position until death, gradually sinking into an apparently comatose condition. More often, convulsions would precede death. Occasionally the animals would suddenly rise from the prone position and run about aimlessly, striking against some object on the floor. This always lasted a few moments only and would end in convulsions and death.

Autopsy findings will be discussed in another place.

It was next necessary to determine the effects of inactivation at 56° C. upon the toxicity of the goat serum.

EXPERIMENTS WITH GOAT SERUM HEATED TO 56° C. FOR TWENTY MINUTES.

These experiments, too, merely corroborate known facts, but they constitute a necessary premise to the work which follows. Therefore a few only will be cited. In each case the serum was tested for its toxicity before inactivation.

Heated Serum from Goat I.—Adult female. The lethal dose of this serum unheated, tested at the same time, was 3.5 c.c. injected intravenously for rabbits weighing from 700 to 800 grams.

Rabbit.—Weight 765 grams. Received 4 c.c. of inactivated goat serum intravenously. No symptoms. Three similar experiments were likewise negative.

Heated Serum from Goat II.—Adult male. The lethal dose of this serum tested on the same day was 4 c.c. for rabbits of similar weight.

Rabbit.—Weight 850 grams. Received 5 c.c. of the inactivated serum intravenously. No symptoms. Two similar experiments were likewise negative.

Heated Serum from Goat III.—Young female. This serum gave severe symptoms in quantities of 4 c.c., and killed in from 8 to 10 minutes when 5 to 5½ c.c. were given.

Rabbit.—Weight 800 grams. Received 4.5 c.c. of the inactivated serum intravenously. No symptoms. Two other experiments with 4.5 and 5 c.c. of this serum were likewise negative.

It is thus clear that inactivation uniformly destroyed the toxicity of the serum.

In connection with the experiments given above, the sera were examined *in vitro* with reference to the action upon washed rabbit corpuscles. In the case of all three of the goats, the fresh serum, tested on the day on which the animal experiments were done, showed marked hemolytic power and definite hemagglutination for washed rabbit corpuscles.

ACTION OF THE GOAT SERA ON RABBIT CORPUSCLES IN VITRO.

Goat I. (*a*) Serum 1 c.c. + rabbit corpuscles 5% 1 c.c. + salt solution 3 c.c = complete hemolysis.
(*b*) Serum 1 c.c. + rabbit corpuscles 10% 1 c.c. + salt solution 3 c.c. = incomplete hemolysis.
Goat II. Serum 1 c.c. + rabbit corpuscles 5% 1 c.c. + salt solution 3 c.c. = incomplete hemolysis.
Goat III. Serum 1 c.c. + rabbit corpuscles 5% 1 c.c. + salt solution 3 c.c. = incomplete hemolysis.

After heating to 56° C. for twenty minutes, the serum no longer showed hemolysis, but the hemagglutinative properties were not noticeably impaired.

From this it appears fair to conclude that, in our experiments, hemagglutination may be ruled out as a cause of the toxicity of the sera.

The serum of the same goat often showed variations, both in hemolytic and in hemagglutinating properties in specimens from bleedings taken a few weeks apart. In one instance—the case of the young female (goat III)—the hemagglutinating property was extremely slight. Nevertheless this serum was fatal to a rabbit of 780 grams in doses of five cubic centimeters.

In the experiments cited above, as well as in a number of similar ones, there appeared to be a rough parallelism between the hemolytic powers of a serum and its toxicity, one cubic centimeter of the serum of goat I, with a minimal lethal dose of two to three cubic centimeters for rabbits of 700 to 800 grams, causing complete hemolysis of a 5 per cent. corpuscle emulsion, while similar quantities of the sera of goats II and III, in whom the lethal dose varied from three and one half to four cubic centimeters but incompletely

hemolyzed this quantity of corpuscles. However, it was noticed that serum which had stood at room temperature for three days, while still hemolytic and hemagglutinating, had often lost much of its toxicity.

Since inactivated serum was without effect upon the rabbits, it seemed necessary to assume that, if there were direct relation between toxicity and hemolysis, either there was no complement free in the rabbit's circulation, or rabbit complement was unable to activate the goat amboceptor against rabbit corpuscles.

The latter assumption was repeatedly put to experimental test as follows:—

(1) Inactive goat serum 1 c.c. + rabbit corpuscles 5% 1 c.c. + rabbit complement 0.2 c.c. = no hemolysis.
(2) Inactive goat serum 1 c.c. + rabbit corpuscles 5% 1 c.c. + rabbit complement 0.5 c.c. = no hemolysis.
(3) Inactive goat serum 1 c.c. + rabbit corpuscles 5% 1 c.c. + rabbit complement 1 c.c. = no hemolysis.
(4) Inactive goat serum 1 c.c. + rabbit corpuscles 5% 1 c.c. + rabbit complement 2 c.c. = no hemolysis.

CONTROLS.

(5) Active goat serum 1 c.c. + rabbit corpuscles 5% 1 c.c. = complete hemolysis.
(6) Anti-sheep amboceptor 3 units + sheep corpuscles 5% 1 c.c. + rabbit complement 0.3 c.c. = complete hemolysis.

(In each case the total volume was made up to 5 c.c. with salt solution.)

It was thus shown that rabbit complement did not activate amboceptor for rabbit cells, an observation analogous to that of Uhlenhuth and Haendel, who found that guinea pig complement did not activate against guinea pig cells.

Similarly, *in vivo:*

A rabbit weighing 820 grams received intravenously 4 c.c. inactivated goat serum + 2 c.c. fresh rabbit serum.

No symptoms resulted; the rabbit remained entirely well.

Thus, rabbit complement did not restore the toxicity of heated goat serum for rabbits.

In connection with the attempted reactivation of goat serum with rabbit complement, control experiments were done in which inactive goat serum was activated *in vitro* against rabbit corpuscles with guinea pig complement. It was found that this could be done, as was to be expected.

REACTIVATION OF GOAT SERUM WITH GUINEA PIG COMPLEMENT.

Heated serum 1 c.c. + rabbit corpuscles 5% 1 c.c. + guinea pig serum 0.2 c.c. +salt solution 3 c.c. = slight hemolysis.

Heated serum 1 c.c. + rabbit corpuscles 5% 1 c.c. + guinea pig serum 0.5 c.c + salt solution 3 c.c. = complete hemolysis.

CONTROL.

Rabbit corpuscles 5% 1 c.c. + guinea pig serum 0.5 c.c. + salt solution 3 c.c. = very slight hemolysis.

Readings were taken after three hours at 37.5° C. and again after six hours at room temperature.

While guinea pig serum in quantities of 0.5 cubic centimeter had slight hemolytic powers of its own for rabbit corpuscles, it was also shown definitely to activate goat serum in these quantities.

It was of interest to note whether inactive goat serum mixed with guinea pig complement would regain its toxicity for rabbits.

The following experiments were done with this point in view.

Rabbit 1.—Weight 615 grams. Received intravenously 4 c.c. of inactivated goat serum together with 1 c.c. of fresh guinea pig serum. No symptoms; remained entirely well.

Rabbit 2.—Weight 565 grams. Received intravenously 4 c.c. of inactivated goat serum together with 2 c.c. of fresh guinea pig serum. No symptoms; remained well.

Rabbit 3.—Weight 740 grams. Received intravenously 4 c.c. of the inactivated goat serum together with 4 c.c. of guinea pig serum. Slightly sick for a short time, but recovered.

CONTROLS.

Rabbit 4.—Weight 620 grams. Received intravenously 4 c.c. of active goat serum. Typical symptoms in 3 minutes. Died in convulsions in 5 minutes.

Rabbit 5.—Weight 680 grams. Received intravenously 4 c.c. of the same serum heated to 56° C. for 20 minutes. No symptoms; remained alive.

Because of the slight illness of the third rabbit, and the fact that the guinea pig serum had moderate hemolytic power for rabbit cells, another control was done in which four cubic centimeters of guinea pig serum, with four cubic centimeters of salt solution, to make up the volume equal to that given to rabbit 3, was injected into a rabbit weighing 820 grams. This rabbit, too, was slightly ill, with apparent difficulty of respiration and weakness for a short time, but recovered entirely within ten minutes and remained well.

It appears, therefore, that although guinea pig serum, *in vitro*, reactivates goat serum against rabbit corpuscles, it does not reac-

tivate the toxicity of goat serum. It is not impossible that this strange phenomenon is due to the slower occurrence of the hemolysis in the case of reactivated serum. But though such a possibility should be considered, the experimental fact seems to point to a definite difference between the hemolytic amboceptor and the toxic agent.

II.

Our preliminary experiments have thus shown that hemagglutination can not be held accountable for the toxic action of the goat serum, and that such toxicity is intimately dependent either upon the hemolytic properties of the serum or, at least, upon some element in the serum which is equally heat-sensitive. In view of Friedemann's success in producing poisons *in vitro* by allowing complement to act upon corpuscles sensitized with immune amboceptor, it seemed promising to examine whether similar toxic substances could be produced, *in vitro,* by the action of the goat serum upon rabbit corpuscles.

It was obviously impossible, in our experiments, to separate complement and amboceptor in the case of the goat serum unless we undertook an extensive search for some complement which would reactivate the toxic property in heated goat serum. Our mode of procedure, therefore, was as follows:—

Measured quantities of fresh goat serum were mixed with large quantities of washed rabbit corpuscles and allowed to stand at 37.5° C. for two to three hours, and following this for two to four hours at room temperature (in one case over night). During this time the mixtures were shaken thoroughly a number of times. At the end of this time free complement and hemolytic amboceptor had disappeared from the supernatant fluid, which was always deeply reddened owing to hemolysis.

Complete absence of complement and of amboceptor for rabbit corpuscles was demonstrated by centrifugalizing and adding a further cubic centimeter of a 10 per cent. rabbit corpuscle emulsion to make sure that no further hemolysis would occur. Some of the supernatant fluid, too, was added to a mixture of sheep corpuscles

sensitized with ten units of anti-sheep amboceptor to make sure that there was no free complement.[2]

When the absence of complement and anti-rabbit amboceptor had been proven, the supernatant fluid was injected into rabbits intravenously.

To make sure that resulting symptoms were not due to the ordinary products of hemolysis, these experiments were controlled by injections of similar and larger quantities of blood laked with distilled water, of a color darker than the supernatant fluid described above.

To insure the experiment still further from the source of error derived from the presence of small numbers of stromata (due possibly to incomplete centrifugalization), controls were injected with masses of the stromata of rabbit corpuscles. The experiments follow.

Experiment I.—Goat I (adult female) was bled at 8:30 A.M. The serum was removed from the clot by centrifugalization one hour later.

The titration of this lot of serum with rabbit corpuscles showed that 1 c.c. did not completely hemolyze 1 c.c. of a 10 per cent. emulsion of rabbit corpuscles.

Three c.c. of this serum were fatal in 5 minutes to a rabbit of 625 grams.

The following mixture was made: 15 c.c. of the fresh serum were added to 15 c.c. of a 50 per cent. emulsion of washed rabbit corpuscles. The mixture was allowed to stand at 37.5° C. for 3 hours and at room temperature for 2 hours. Centrifugalization yielded a claret red supernatant fluid and a massive sediment of unhemolyzed corpuscles. Further addition of 1 c.c. of 10 per cent. rabbit corpuscles gave no further hemolysis (as controlled by the addition of a similar quantity of rabbit corpuscles to blood laked by distilled water of the same color as the supernatant fluid).

One and one half c.c. of the supernatant fluid was taken to represent approximately 1 c.c. of the original serum.

Intravenous injection of the supernatant fluid into rabbits yielded the following results:—

Rabbit 1.—Weight 765 grams. Received intravenously 4.5 c.c. of the supernatant fluid. Marked symptoms in 2 minutes. Dead in 4 minutes.

Rabbit 2.—Weight 1,030 grams. Received intravenously 4.5 c.c. of the same supernatant fluid. Symptoms in 4 minutes. Dead in 7 minutes.

[2] The latter test was actually of little value, since it was found that normal goat serum is but weak in complement for sensitized sheep corpuscles. The former test, however, was sufficiently conclusive for our purposes.

CONTROLS.

Rabbit 3.—Weight 625 grams. Received 3 c.c. of the original serum made up to 4.5 c.c. with salt solution. Symptoms in 2 minutes. Dead in 4 minutes.

Rabbit 4.—Weight 600 grams. Received original serum, inactivated, 3.5 c.c. made up to 4.5 c.c. with salt solution. No symptoms.

Rabbit 5.—Weight 745 grams. Received 5 c.c. of blood hemolyzed with distilled water, of a color slightly darker than the supernatant fluid injected into rabbits 1 and 2. No symptoms.

Rabbit 6.—Weight 760 grams. Received 2 c.c. of the sediment of stromata resulting from the laking of rabbit's blood with distilled water, the volume being made up to 4.5 c.c. with salt solution. Seemed a little uncomfortable immediately after the injection but was perfectly well within 3 minutes.

Experiment II.—Serum of goat I, taken on day of experiment, causes almost complete hemolysis of 1 c.c. of a 10 per cent. emulsion of rabbit corpuscles.

Fifteen c.c. of the goat serum were added to 15 c.c. of a 25 per cent. emulsion of washed rabbit corpuscles. The mixture was allowed to stand for two hours at 37.5° C., and following this for 2 hours at room temperature. After centrifugalization the transparent red supernatant fluid was pipetted away and a part of it tested for free amboceptor and complement as above, with negative result.

Rabbit 1.—Weight 540 grams. Received intravenously 5 c.c. of the supernatant fluid. Symptoms in 3 minutes. Dead in 5 minutes.

Rabbit 2.—Weight 600 grams. Received intravenously 4.5 c.c. of the supernatant fluid. Symptoms in 3 minutes. Dead in 6 minutes.

Rabbit 3.—Weight 650 grams. Received intravenously 4 c.c. of the supernatant fluid. Symptoms in 1 minute. Dead in 2¾ minutes.

CONTROLS.

Rabbit 4.—Weight 655 grams. Received 4 c.c. of the original serum. Symptoms in 3 minutes. Dead in 12 minutes.

Rabbit 5.—Weight 695 grams. Received 5 c.c. of laked rabbit's blood (by distilled water) darker in color than the supernatant fluid used above. Slightly increased respirations for a few minutes, then normal.

Rabbit 6.—Weight 615 grams. Received intravenously 5 c.c. of laked blood as in rabbit 5. No symptoms.

Experiments like the ones given above were successful only when the goat serum had been taken not longer than twenty-four hours before the experiments. On two occasions, when the goat serum had stood for forty-eight hours, experiments similar to those just described, gave, in one case, weak toxic effects only, and in the other, they were entirely negative.

The experiments cited above represent the most striking demonstrations which we were able to obtain. In a number of other

experiments, with slightly varied technique, and in some with similar technique, the toxicity of the supernatant fluid was less marked but nevertheless quite distinct.

An example of such an experiment follows:—

Experiment III.—Serum of goat I. Fourteen c.c. of the fresh serum were mixed with 10 c.c. of concentrated washed rabbit corpuscles. The mixture was shaken up and allowed to stand at room temperature over night. Although marked hemolysis had taken place, the mixture was again exposed to 37.5° C. for one hour on the following morning. After this it was centrifugalized and the supernatant fluid decanted.

Rabbit 1.—Weight 900 grams. Received 3.5 c.c. of the supernatant fluid intravenously. Became severely sick in 2 minutes. Remained so for 30 minutes; then gradually recovered.

Rabbit 2.—Weight 1,000 grams. Received 5 c.c. of the supernatant fluid intravenously. Grew distinctly sick in 10 minutes. Seemed to recover, but was found dead on the following morning.

Rabbit 3.—Weight 950 grams. Received 6 c.c. intravenously. Extremely sick and fell to side in 2 minutes. Died in 4 minutes.

CONTROLS.

Rabbit 4.—Weight 960 grams. Received 5 c.c. of laked rabbit's blood (by distilled water). No symptoms.

Rabbit 5.—Weight 850 grams. Received 3 c.c. of sediment of stromata from blood laked by distilled water. Slight respiratory discomfort for a few moments; then perfectly well.

It seemed to us important to determine whether or not the quantities of supernatant fluid necessary to kill corresponded to or were larger or smaller than the amounts of original serum represented in them. For this purpose the following experiment was done.

Experiment IV.—Serum of goat I. Ten c.c. were mixed with 10 c.c. of a 25 per cent. emulsion of washed rabbit corpuscles. The mixture was allowed to stand at 37.5° C. for 4 hours. At the end of this time 2 c.c. of 50 per cent. rabbit corpuscle emulsion were added, and no further hemolysis was noted after observation for one half hour. The mixture was then centrifugalized for 20 minutes, after which there was a tightly packed sediment of cells and stromata measuring 2 c.c. and a supernatant fluid of nearly 18 c.c. (The discrepancy was probably due to spilling during manipulation and loss in transfer to the centrifuge tubes).

As nearly as we could estimate, then, the supernatant fluid contained in each 1.8 c.c., about 1 c.c. of original serum.

Rabbit 1.—Weight 525 grams. Received 5 c.c. of the supernatant fluid. Died in convulsions in 2 minutes.

Rabbit 2.—Weight 740 grams. Received 4 c.c. of the supernatant fluid.

Very sick in 3 minutes. Urinated. Front legs paralyzed in 6 minutes. Lay flat without moving for 15 minutes; then gradually recovered.

Rabbit 3.—Weight 575 grams. Received 3.6 c.c. of the supernatant fluid. Distinctly sick in 3 minutes. Very sick in 6 minutes; staggered and lay prone with respiratory distress. Very sick for 30 minutes; then recovered.

Rabbit 4.—Weight 620 grams. Received 2 c.c. of the original serum, corresponding to the amount of serum in 3.6 c.c. of the supernatant fluid. Distinctly sick in 10 minutes. Remained so for thirty minutes; then recovered.

Rabbit 5.—Weight 700 grams. Received 2.8 c.c. of the original serum, corresponding to the amount of serum represented in 5 c.c. of the supernatant fluid. Very sick in 3 minutes. Remained so for 30 minutes and fell on its side; outcome doubtful for one hour, but gradually recovered.

It appears from this experiment that the toxicity of measured quantities of the supernatant fluid, corresponds, with fair accuracy, to the toxicity of the amounts of original serum contained in it. This, incidentally, precludes the possibility of attributing the toxicity of the supernatant fluid of our mixtures to traces of unabsorbed hemolytic amboceptor or complement remaining in it.

It was found, furthermore, that heating the supernatant fluid of goat serum absorbed with rabbit corpuscles to 56° C. destroyed its toxic properties, as it did those of the original serum. This is illustrated in the following protocol.

Rabbit 1.—Weight 745 grams. Received 4.5 c.c. of supernatant fluid, as in the preceding experiment. Died in 4 minutes.

Rabbit 2.—Weight 700 grams. Received 4.5 c.c. of the same supernatant fluid heated to 56° C. for 20 minutes. No symptoms.

Rabbit 3.—Weight 750 grams. Received 5 c.c. of heated supernatant fluid. No symptoms.

We may summarize the experimental facts so far obtained as follows:—

Normal goat serum is toxic for rabbits, when intravenously injected, in doses varying from two to five cubic centimeters. *In vitro,* the goat serum hemolyses and hemagglutinates rabbit corpuscles. When heated to 56° C., the goat serum loses its toxic and hemolytic properties but not its hemagglutinating property.

The addition of fresh rabbit complement to inactivated goat serum reactivates neither hemolysis nor toxicity. The addition of fresh guinea pig complement, however, reactivates the hemolytic but not the toxic property.

When fresh, toxic goat serum is mixed with a large excess of washed rabbit corpuscles until no further hemolysis takes place and until all determinable traces of amboceptor and complement for rabbit cells have disappeared, the supernatant fluid of such mixtures is toxic in the same way as the original serum. This toxicity does not depend upon the products of laked blood in it, nor upon unremoved stromata. The toxicity, also, is proportionate to the amount of original serum contained in the injected quantity of supernatant fluid and is destroyed by heating to 56° C., as was the toxicity of the original serum.

These experimental facts permit us to conclude that the toxic action of the goat serum upon rabbits does not depend on its hemagglutinins for rabbit corpuscles, and that this toxicity is directly dependent upon the coöperation of a heat-sensitive body in the serum.

Whether or not we can identify the toxic factors with the hemolysins in the serum is difficult to decide. Our experiments in which goat serum was exposed to an excess of rabbit corpuscles and found toxic after complete removal of hemolytic amboceptor or complement permit of a twofold interpretation. On the one hand, we may assume that the hemolytic serum acting upon the erythrocytes has produced from them a toxic body analogous to the poisons obtained in Friedemann's experiments by the action of immune amboceptor and complement upon red cells, and belonging to the category of Friedberger's (8) "anaphylatoxins."

On the other hand, it is not impossible that the removal of hemolytic amboceptor and complement from the goat serum, as in our experiments, may have left unchanged in the serum a heat-sensitive toxic body, either simple or as an amboceptor-complement complex, independent of hemolysins. The writer believes the latter state of affairs to be more probable for the following reasons:—

1. Attempted reactivation of the toxic action of heated goat serum with guinea pig complement was unsuccessful, while its hemolytic properties were thus easily reactivated.

2. The toxicity of the goat serum seemed to disappear more rapidly on standing than did its hemolytic properties.

3. The quantities of original serum represented in toxic doses

of supernatant fluid from mixtures of cells and serum (in which hemolytic complement and amboceptor have been used up) corresponded with considerable accuracy to toxic doses of the original serum.

4. The toxicity of the supernatant fluid of the mixtures of serum and cells above described, freed of hemolytic complement, was destroyed by heating to 56° C.

We may sum up this part of the work briefly, then, by stating that the toxic action of goat serum for rabbits depends upon a heat-sensitive factor independent of hemagglutinins and probably of hemolysins. Whether this toxic factor is a preëxisting heat-sensitive poison or whether it is a complex mechanism of complement and amboceptor, producing a toxic substance by action on rabbit proteid, can not at present be decided. The parallelism of toxicity and hemolytic properties of sera, together with certain analogies of these phenomena with those observed by Friedemann (where immune amboceptor and complement, acting on red blood cells produced poisons before actual hemolysis took place), cause us to favor the latter view. It may be suggested that possibly we are dealing with a substance like the hypothetical "albuminolysin" of Nicolle (9).

This thought is further strengthened by the experience of Battelli in which, it will be remembered, the toxic bodies resulted from the action of immune sera not on red blood cells but on red blood cell extracts.

III.

The question also arises whether the phenomena observed by us may be classed with true anaphylaxis. Friedemann in his work on the toxic substances liberated from erythrocytes by immune sera, regards the phenomena observed by him as beyond question anaphylaxis, and Doerr, casually referring to the toxic action of normal sera expresses the belief that this, too, must be regarded as anaphylactic in nature. Kraus (10), on the other hand, is unwilling to accept this interpretation.

While we believe that it is extremely probable that the phenomena following the injection of normal toxic sera into rabbits

and other susceptible animals are closely allied to true anaphylaxis, we do not think that, at present, there is positive proof of such an identity. It appeared of value, therefore, to work out for the condition studied by us some of the phases which may eventually help in deciding its relation to anaphylaxis.

1. *Autopsy Findings.*—None of the rabbits killed either by normal serum or by the products formed in the test tube experiments showed the inflated lungs described for typical anaphylaxis in guinea pigs by Gay and Southard (11), Auer and Lewis (12), and others. In all cases, if the animals were examined immediately after death, the lungs were found to be empty, but the heart was still weakly beating and continued so often for several minutes. Coagula were found in the right auricle in two cases only, in which the animals had died within less than four minutes. When the animals lived for longer than this, blood coagulation was not present when immediate examination was carried out. In a number of these animals, careful dissection of the larger pulmonary vessels immediately after death revealed no clots. In fact, in a number of cases the blood observed in the mesenteric veins could be seen to remain fluid for as long as twenty to thirty minutes after death. What relation blood coagulation has to this condition in our cases, must be left undecided. However, we do not believe that coagulation can be justly regarded as the immediate cause of death except, possibly, in the few cases in which death occurred in less than three or four minutes. The complete and rapid recovery of underdosed animals would also argue against this. In a few cases, hemoglobinuria and transudation of hemolytic serum into the peritoneal cavity were observed. Other gross changes were absent. Histological examinations were not made.

2. *Fall of Temperature.*—The characteristic fall of temperature described by Pfeiffer (13) has indeed been present in our cases, but this, inasmuch as our injections were necessarily made with active serum, has little significance, since Ranzi (14) has shown that normal sera may produce rapid temperature depression in normal animals on first injection. Drops of from two to three degrees were often noticed in our cases within twenty minutes.

3. *Diminution of Complement.*—The diminution of complement

after injection of normal sera into animals has been noted by Doerr and Moldovan. It is an occurrence which we would not expect since, in the mechanism studied by us, the complement of the rabbit seems to take no part in the resulting reaction. Nevertheless, the complement was measured a number of times with very uniform result. Two of these titrations were as follows:—

COMPLEMENT TITRATION BEFORE AND AFTER INJECTION OF NORMAL GOAT SERUM.

A rabbit weighing 895 grams was bled from the ear just before injection and was given intravenously 3.5 c.c. of fresh normal goat serum. It became severely sick after eight minutes and gradually grew worse during the course of an hour. At the end of this time, blood was taken from the carotid. Complement titration was carried out by adding increasing quantities of the rabbit serum to sheep corpuscles activated with 6 units of anti-sheep amboceptor.

Complement.	Hemolysis before Injection.	Hemolysis One Hour after Injection
0.1 c.c.	Very slight	None.
0.2 c.c.	Distinct.	Very slight.
0.3 c.c.	Almost complete.	Distinct.
0.4 c.c.	Complete.	Almost complete.
0.5 c.c.	"	Almost complete.
0.6 c.c.	"	Complete.
0.7 c.c.	"	"
0.8 c.c.	"	"
0.9 c.c.	"	"
1.0 c.c.	"	"

A similar experiment resulted as follows:

Complement	Hemolysis before Injection.	Hemolysis One Hour after Injection.
0.1 c.c.	Very slight.	No hemolysis.
0.2 c.c.	Distinct.	Very slight.
0.3 c.c.	Almost complete.	Distinct.
0.4 c.c.	Complete.	Almost complete.
0.5 c.c.	"	Complete.
0.6 c.c.	"	"
0.7 c.c.	"	"
0.8 c.c.	"	"
0.9 c.c.	"	"
1.0 c.c.	"	"

In view of the large quantities of rabbit complement necessary for activation of the complex, it did not seem necessary to use complement dilutions.

In all cases there was a distinct diminution of complement, in spite of the non-participation of the complement of the subject in the reaction. Yet this diminution was proportionately less than that observed in the case of true anaphylaxis.

4. *Anti-Anaphylaxis.*—Rabbits which had recovered from sublethal doses of goat serum showed no increased resistance to subsequent injections. This was observed even after two or three injections had been given on the days immediately preceding. No experiments were made to ascertain whether or not prolonged treatment would immunize. It appears certain, however, that nothing truly akin to anti-anaphylaxis takes place. This fact, like some others, is given for record, though it is, of course, understood that anti-anaphylaxis even in typical experiments is of uncertain occurrence in rabbits.

5. *Fall of Blood Pressure.*—The blood pressure of rabbits injected with goat serum was taken in the usual manner from the carotid artery. The results showed in all cases a preliminary rise of pressure followed in large animals given moderate sublethal doses by a slight fall, and soon returning to normal. When younger rabbits were given lethal doses, sufficient in amount to kill within six to seven minutes, the preliminary rise of blood pressure was of short duration, followed within two or three minutes by a rapid falling of the pressure progressively continuing until death.[3]

6. *Etherization.*—Ether narcosis did not, in any of our experiments, prevent death when lethal doses of goat serum were given.

7. *Atropin.*—This did not prevent death nor did it seem to reduce the speed or violence of symptoms.

The preceding experiments are cited simply because the writer believes that they may aid in more accurately defining the phenomena following the injection of toxic normal sera into susceptible animals. It does not seem justifiable, on available evidence, however, to assume, at present, an identity of these phenomena with anaphylaxis. While such a possibility must be considered, it would seem that more proof should be brought forward before the two

[3] We are indebted to Professor Albert C. Crawford for advice in carrying out these tests.

conditions can be fully identified, as has been done by some recent writers.

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STUDIES IN IMMUNITY AND ANAPHYLAXIS.

The Proteins of the Kidney and Liver.*

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The investigations of the past eight years, having for their object the demonstration of antisera for the nucleoproteins of various animal cells, have not given uniform results (1). For this reason, and also because the earlier work of one of us (Pearce) on this subject has been criticised, the present investigation was undertaken. The scope of the work has been somewhat broadened, in that an attempt has been made to produce antisera for globulins and albumins as well as for nucleoproteins. Further, in order to determine specificity, the same proteins have been used in experiments on anaphylaxis for the reason that by that method the question of relative specificity, or "special action," could more readily be determined. As most of the previous investigations were concerned with antisera for the liver and kidney, the work has been limited to the proteins of these organs.

METHODS.

The organs were rendered free of blood *in situ* by prolonged washing through the blood vessels. By placing a ligature about a cannula in the aorta at the level of the diaphragm and making an opening in the vena cava, a continuous flow of warm 0.85 per cent. salt solution was passed through the liver and both kidneys simultaneously. As the operation was done under ether anesthesia, the washing frequently was well under way before the death of the animal took place. Nine to twelve liters of salt solution were necessary to blanch the organs completely. Bile and urine were also removed to some extent by severing the bile ducts and ureters. The organs were hastily re-

*Aided by a grant from the Rockefeller Institute for Medical Research, New York. Received for publication, May 13, 1911.

moved, and the fat and coarser connective tissue having been dissected away, were passed quickly through a hashing machine. The resulting mass, after the addition of infusorial earth and sand, was pressed in a Buchner press. Two volumes of 0.85 per cent. salt solution and a little chloroform were added to the combined press juice and tissue pulp and the mixture was placed in a cold storage compartment at a temperature varying from $-1°$ to $+2°$ C. After eighteen to twenty-four hours, the coarser particles having been removed by straining through gauze, the residue was centrifuged to remove suspended particles. From the fluid thus obtained, the nucleoproteins were precipitated by acetic acid, which after eighteen to twenty-four hours rest in cold storage were removed by centrifugalization. The nucleoproteins were first repeatedly washed with 0.85 per cent. salt solution and then dissolved in it after the addition of sodium carbonate. This process of precipitation, centrifuging, washing, and solution was repeated two or three times, and the final precipitate was dissolved in 0.5 per cent. sodium bicarbonate. If not used immediately it was kept at a temperature close to freezing point. From the fluid remaining after the first precipitation of the nucleoproteins by acetic acid, the globulins and albumins were obtained after neutralization with sodium bicarbonate. Ammonium sulphate was added to half saturation, and after eighteen to twenty-four hours in cold storage the precipitate (globulins) was filtered off and dissolved in distilled water to which a little sodium bicarbonate had been added. This process was repeated once and the final solution allowed to dialyze in running water for from four to six days. It was then made alkaline with sodium bicarbonate, filtered, and kept in cold storage until used.

From the filtrate of the half saturated solution the albumin portion was obtained by complete saturation with ammonium sulphate. The precipitate was dissolved in distilled water and allowed to dialyze in running water for from four to six days. It was then filtered and placed in cold storage.

The separation of the globulin and albumin precipitates was hastened by suction filtration and by the use of the centrifuge. Except during the relatively short periods of filtration, the solutions were kept at a temperature close to the freezing point. As fresh preparations were made for each series of injections, the period elapsing between the completion of the preparation of the product and the time of injection was, with one exception, never more than twenty-four hours, and usually less than one hour. This one exception occurred in the liver series in which the same lots of nucleoprotein, globulin, and albumin were used for the fourth and fifth injections of each of these substances. The presence of nucleoprotein in the preparation known by that name was determined by demonstrating the presence of the purin bases by Kossel's method, which was considered a more satisfactory criterion than the phosphorus content

The various fractions were injected into rabbits intraperitoneally, at intervals of five to seven days. A total of five injections was given, after which the animals were bled at the expiration of a period of at least seven days after the final injection. It seemed best, in order to keep the preparation in as natural a condition as possible, to estimate the amount injected as the equivalent of so many grams of moist liver or kidney substance. Thus, for example, when the total globulin yield of 120 grams of liver was injected into four

rabbits in equal quantities, we have considered each as receiving the globulin equivalent of 30 grams of liver substance. On this basis, all animals, in both kidney and liver series, received, at the first injection, nucleoprotein, globulin, or albumin equivalent to 20 grams of moist tissue with a gradual increase to an equivalent of 40 to 50 grams at the final injection.

RESULTS.

Tests in Vitro.—The results obtained by testing the activity of the sera *in vitro* are given in tables I and II. Much stress has been laid on the presence of agglutinins and precipitins in nucleoprotein sera as evidence of special or specific action. Although such substances may be present, they do not, if we are to follow the rules of bacterial immunization, indicate cytotoxic activity, any more than a fluid agglutinating the typhoid bacillus is to be considered as bactericidal. The tests were made with eighteen sera representing three rabbits of each of the six types of immunization; only one of each type, however, is given in the tables.

The manner of making the tests did not differ materially from that generally employed, except in some minor details.

In testing for agglutinins for organic cells, the washed organs were ground with a hashing machine, suspended in two volumes of 0.85 per cent. sodium chlorid solution, and centrifuged at high speed (about 2,000 revolutions per minute) until three distinct strata could be seen. It was found that the middle stratum was most satisfactory. To one cubic centimeter of such a suspension, 0.1 c.c. of the antiserum was added, the mixture shaken and allowed to stand at room temperature. Agglutination of the red blood corpuscles was determined by adding 0.1 c.c. of the serum to 1 c.c. of a 5 per cent. suspension of unwashed dog's blood. This unwashed blood was used in order to approximate the conditions existing in the body in the injection experiments to be described later. Hemolysis was determined in the tubes used for hemagglutination. In the precipitation tests, 1 c.c. of the various solutions of globulins, nucleoproteins, and albumins was mixed with 0.1 c.c. of immune serum. This means that the serum was used in a dilution of 1 to 11. This dilution was followed throughout, but other dilutions were used with selected sera, as will be mentioned later.

The various solutions of nucleoproteins, globulins, and albumins, used in the precipitation tests, were similar in every way to those used for injection. Their reaction to litmus was neutral or slightly alkaline—as nearly neutral as the method of preparation would allow—but never acid.

Sera were used within forty-eight hours after withdrawal from

the rabbits, except that the same specific hemolytic serum was used for all the experiments. The results were usually controlled by independent readings by at least two persons.

As will be seen by reference to the accompanying tables (tables I and II), the results were controlled by a specific hemolytic serum, a normal rabbit serum, and by salt solution. The hemolytic serum used was prepared by making five injections, at intervals of five days, of five cubic centimeters of unwashed defibrinated dog's blood into the peritoneum of the rabbit. The normal rabbit serum was always drawn at the same time as the sera to be tested. The time of reading was as near one half hour, one hour, two hours, and four hours as convenience would permit. It was found that the results at the end of twenty-four hours were usually identical with those after four hours, and for this reason the later readings are not included in the charts.

Table I, which presents examples of the experiments with the different types of kidney sera, shows that the sera exert a rapid agglutinating effect on the kidney cell suspension and no effect on the liver cell suspension. That this is not specific is shown by the fact that the anti-liver sera as well as normal rabbit serum produce exactly the same effect. It appears to be a matter of agglutinability of cells rather than of the agglutinating action of the sera. The same general statement is true of the agglutination of red blood corpuscles. Two other sera in each of these three groups were tested with similar results as to agglutination, except that one antiglobulin serum failed to agglutinate kidney cells.

The sera are very slightly hemolytic except when incubated, under which circumstances one serum of each group showed moderate hemolyzing activity after two to four hours.

With regard to precipitation, it is seen that no serum has a precipitating action on dog serum analogous to that of the hemolytic immune serum. This held with the other sera tested, except in the case of one anti-albumin serum.

The anti-nucleoprotein serum presented in the table shows a late precipitating action on all the albumin solutions. This is characteristic of this type of serum, though the action was more rapid in one

of the sera not presented. The anti-globulin serum is entirely inactive, while the anti-albumin serum is highly active throughout. The only suggestion of relative specificity was seen in the case of one anti-globulin serum and one anti-albumin serum which precipitated the kidney solutions somewhat sooner than the liver solutions. Attempts to show a more definite specificity by the use of higher dilutions (1 : 100, 1 : 500, 1 : 1000) were fruitless, the sera not precipitating in these dilutions. The hemolytic serum does not act more powerfully on one group of protein than on the other.

In considering table II, in which the experiments with anti-liver sera are presented, it should be said that the statements made in the discussion of table I hold true as regards the agglutinating action of the sera on organ cells; that is, kidney cells and not liver cells are agglutinated. These sera are much less active agglutinators of the red blood corpuscles, however, than the anti-kidney sera, and are practically free from hemolytic activity.

The absence of precipitating action on dog serum, as shown in the table, was not uniform, as it occurred in a slight degree in one of three sera of each type. The hemolytic serum appears to be more active in this series than in the anti-kidney series, probably because it is somewhat older. As in the kidney series, higher dilutions of the anti-liver sera were tried, but no degree of specificity was observed.

It seems safe to conclude that the specific organic precipitating power of any of these sera is at best slight and relative only. Moreover, a similar power has been demonstrated in the anti-globulin and anti-albumin sera rather than in the anti-nucleoprotein sera. Absolutely no organ specificity is demonstrable as regards the agglutination experiments, since agglutination appears to depend on agglutinability of the cells rather than on specific properties of the sera. Hemolytic activity is absent in all the sera except after incubation, when it puts in a delayed appearance of slight degree.

Tests in Vivo.—The effect of the sera on living animals was determined by intravenous and intraperitoneal injections into dogs. All injections were in the proportion of two cubic centimeters of serum per kilo of body weight. The urine of all the animals was

carefully studied, as were sections from the liver and kidneys. Dogs suffering from spontaneous nephritis, or other conditions causing albuminuria, were excluded. As experience has led us to view with suspicion findings based on the examination of urine passed in the ordinary animal cages, we have kept all animals in metabolism cages, fed on a dog biscuit diet, and have used only female dogs, in order that the presence of coagulable protein in a "cage" urine might be controlled by the examination of a specimen obtained by catheterization. This precaution is most important and has transformed several apparently positive results into negative ones. Foreign coagulable protein in the urine as the results of vomiting, diarrhea, bloody vaginal discharge, and oozing from a wound have in each instance been detected by comparison with a catheterized specimen, whereas without the latter the result would have been considered positive or doubtful. Oozing of serum or blood from the wound necessary for intravenous injection has been prevented by utilizing a small vein of the lower leg, where a tightly fitting gauze dressing and bandage can be applied. All injections were made under ether anesthesia. The urine was examined by the heat and acetic acid test and with acetic acid alone for a control; to doubtful urines the ferrocyanid test was also applied.

Kidney Nucleoprotein Serum.—Six dogs received serum from four different rabbits. In two, the injection was intraperitoneal, and in the others, it was intravenous. Vomiting occurred immediately in the animals injected intraperitoneally, but not in those injected intravenously. It was transient in character and the animals, with the exception of two which developed diarrhea, remained in excellent condition. All were chloroformed at the end of from five to ten days. In one, a flocculent precipitate occurred in the "cage" urine on the first day, increasing to one eighth gram, by Esbach's method, on the third day, but on each of these days the catheterized specimen showed no coagulable protein. The source of this could be ascribed to oozing from the operation wound, the dog having torn away the bandage each night. In a second animal, the occurrence of a slight flocculent precipitate in the "cage" urine, not confirmed by the catheterized specimen, was traced to a previously unobserved mild diarrhea. In a third animal, the urine was negative for two days, but after a second injection of serum, a fine flocculent precipitate appeared on the third day, but was not detected on the three succeeding days. The urine of the remaining three animals was free from albumin.[1]

[1] Statements concerning the presence of traces of coagulable protein, in the dog's urine must always be viewed with caution. In normal dogs, it is customary

Kidney Globulin Serum.—Six dogs were injected with this serum, four intravenously and two intraperitoneally. The urine in two of the former was free from albumin for five and seven days respectively; in a third, coagulable protein appeared on the day following injection and persisted until the third day when the animal was chloroformed. The amount of albumin for each day by Esbach's method equalled one fourth gram. The catheterized urine on the third day was strongly ammoniacal; and at autopsy a hemorrhagic cystitis was found. The interpretation of the albuminuria, therefore, was doubtful. The "cage" urine of a fourth animal, receiving two injections, the second of which was after an interval of four days, contained coagulable protein intermittently; but repeated catheterization showed none in the bladder urine. The coagulable protein in the "cage" urine was found to be due to contamination by diarrheal stools. Of the animals receiving the serum intraperitoneally, one developed albuminuria on the day following the injection. This persisted, equalling from one fourth to three eighths gram by Esbach's method, for four days, when the animals were chloroformed. No adventitious cause for the presence of albumin could be discovered. Upon histological examination, the kidneys of this animal show moderate granular degeneration of the tubular epithelium with an occasional granular cast in the collecting tubules. The second animal which received the same treatment showed no albumin for a period of eight days.

Kidney Albumin Serum.—Four animals were injected, of which three received the serum intravenously. One showed no albumin; the "cage" urine of the other two contained coagulable protein, which, however, was not present in the catheterized urine, and was made out to be due to a slightly bloody vaginal discharge. One of the two received two injections at two day intervals. The fourth animal, injected in the peritoneal cavity, developed albuminuria after twenty-four hours, which persisted until the eighth day, when the animal was chloroformed. By Esbach's test, the albumin varied from one fourth to one half gram. No cause other than kidney injury could be found for this albuminuria. Upon histological examination, the convoluted tubules show granular and fatty degeneration with much desquamation and, in the collecting tubules, an occasional fine granular cast.

The results in the above experiments are based on single injections, and in at least one animal of each series, on a double injection of the serum after an interval of two or four days. Still another experiment made for the purpose of comparing the action of the three different sera on the same animal, yielded negative results. Nucleoprotein, globulin, and albumin sera were successively injected intravenously at intervals of four days, and the animal was chloroformed six days after the last injection without producing albuminuria.

Liver Nucleoprotein Serum.—Seven animals were injected, four intravenously

to obtain a slight turbidity on adding acetic acid or potassium ferrocyanid, which after standing for twenty-four hours settles to the bottom of the tube as a faint fluffy cloud. This turbidity is due, in all probability, to proteins of the mucin and nucleo-albumin type derived from the collecting tubules, which are normal constituents of dog's urine. It has therefore been disregarded, and we have considered as evidence of kidney injury only a flocculent precipitate or a turbidity produced by acetic acid which was greatly increased by the application of heat.

and three intraperitoneally. Two of the first and three of the latter group showed no change in the urine, but one of the second group developed a severe atack of vomiting shortly after injection and died on the second day from acute intestinal obstruction due to intussusception. The two remaining animals of the series developed albuminuria on the day following injection. In one, the albuminuria on the second day equalled one fourth gram by Esbach's test and then began to diminish; in the other it equalled seven eighths gram on the fourth day, when the animal was employed for oncometric studies. Both animals were injected intravenously and had transient vomiting shortly after receiving the serum. No cause for the albuminuria, other than kidney injury could be found. The kidney of both these animals showed moderate granular and vacuolar degeneration with occasional casts.

Liver Globulin Serum.—In this series alone was a lethal effect observed. A dog under ether anesthesia receiving serum intravenously, in the proportion of 1 c.c. to 600 grams of body weight, died within five minutes. To eliminate the possibility of death from ether, the same serum was injected intraperitoneally in the same dose into two other animals. Both vomited excessively, showed evidence of collapse, and died after three and four hours respectively. At autopsy the spleen in each was intensely congested, and in one, a few small hemorrhages were found in the gastric mucosa. The blood in the vessels showed in each evidence of laking, and the urine gave a slight, but definite, flocculent precipitate by the heat and acetic acid test. The effects are suggestive of a powerfully hemolytic serum, but as all the serum had been used for injection, no *in vitro* tests could be made. The sera of three other rabbits in this series failed to produce this acute effect, and there was no renal disturbance during periods of five to eight days after either single or double injections given either intravenously or into the peritoneal cavity.

Liver Albumin Serum.—Seven dogs were injected. Vomiting was observed in each of two animals receiving an intraperitoneal injection but not after intravenous injection in the other five animals. The urine of six showed no albumin; the seventh showed albumin after twenty-four hours, which persisted until the third day when the animal was used for oncometric studies. The albumin reached three fourths gram by Esbach's test, and the urinary sediment contained a few fatty epithelial cells but no definite casts. Section of the kidney showed extreme granular and fatty degeneration but no casts.

To supplement the single and double injections, as in the kidney series, one animal was given intravenously, in succession, the three types of liver sera. The globulin serum followed by the albumin serum produced no changes in the urine; on the day following the administration of the nucleoprotein serum, which was given last, a hemorrhagic diarrhea developed and the animal died on the third day. At autopsy an extensive hemorrhagic colitis was found. The catheterized urine of the second and third days showed only a faint turbidity by the heat and acetic acid test.

DISCUSSION.

From the physiological reactions presented, it is evident that aside from the lethal effect of one globulin serum, the only evidence

of toxicity is presented by the somewhat frequent gastro-intestinal disturbances, vomiting, and diarrhea produced, and an occasional mild albuminuria. As albuminuria develops as often with liver as with kidney sera, the several disturbances appear to indicate an effect on the organs of elimination rather than a special action on the organs for which the sera are supposed to have a special affinity. The theory that the cell nucleoprotein is the essential substance in the production of a cytotoxic serum cannot be supported in view of the fact that a serum prepared by the injection of nucleoprotein is no more toxic than sera prepared from the globulins and albumins of the same organs.

Furthermore, the histological examination of the livers and kidneys of the injected animals gives no support to the view of "specific" or "special" action. The kidneys of animals receiving either liver or kidney sera showed the same general condition of moderate granular and vacuolar degeneration of the epithelium. Glomerular lesions were entirely absent except in animals killed by the peculiarly toxic liver-globulin serum. Abundant cast formation is never seen in sections, but a few casts were once found in the kidney nucleoprotein series, twice in the kidney globulin, and twice in the kidney albumin series. On the other hand, casts occurred seven times in the liver series. A comparison of the histological changes in the kidneys of animals receiving the various types of sera shows but little variation. The same moderate degenerative lesions are produced by liver and kidney sera alike, and represent apparently a common effect arising in the process of elimination through the kidney.

Moreover, the liver failed to show a relation between the type of serum injected and the histological changes present. Those cells of the liver which were not normal showed more or less granular and vacuolar degeneration, but this was not more frequent after the injection of liver serum than after kidney serum. Focal necroses were never seen.

ANAPHYLAXIS.

The failure to obtain by the methods of immunization antisera having special action led to the use of the methods of anaphylaxis.

The specificity of the anaphylaxis reaction in so far as the proteins of different animals are concerned is now a generally accepted fact, but organ specificity is by no means certain. Kraus, Doerr and Sohma (2), Andrejew (3), Uhlenhuth (4), and Pfeiffer and Mita (5) have clearly demonstrated the specificity of the anaphylaxis reaction for the proteins of the crystalline lens. Such anaphylaxis is a crossed reaction for the lenses of different species but not for serum, which fails to interact with the lens extract either as a sensitizer or as an intoxicating body. Pfeiffer and Mita, Thomsen (6), and Doerr and Moldovan (7) claim to have shown, by both active and passive anaphylaxis, a qualitative difference in reaction to erythrocyte proteins and serum proteins. Pfeiffer and Mita extend this observation to the differentiation of white and yolk of egg. Ranzi (8) has made an extensive study using the liver, kidney, spleen, and ovary, and has reached the conclusion that these give a species reaction but no evidence of organ specificity. Pfeiffer (9) objects to Ranzi's experiments on the ground that the organs used were unwashed and therefore could not be expected to show specificity either as sensitizer or intoxicator because of the natural content of serum protein. He did not repeat the experiments with all the organs that Ranzi used but confined his work to the crossed reactions with extracts of the kidney, spermatozoa, erythrocytes, and ox serum. He estimates, according to his elaborate formula, that the animals sensitized to a given organ extract respond more markedly to that extract than to the extracts of other organs, and he therefore claims a definite organ specificity. He admits, however, that the animals sensitized to kidney react very considerably to the proteins of spermatozoa, but he thinks that this is due to the close embryonic origin of kidney and testicle. In spite of his conclusions, an examination of his charts shows that the so-called specificity is not absolute but merely relative.

METHODS.

Our experiments differ from those of previous investigators in that instead of working with organ extracts we used the same protein fractions (nucleoprotein, globulin, and albumin) as in the immunization experiments. The protein solutions were of such concentration that one cubic centimeter contained the nucleoprotein, globulin, or albumin of one gram of organ substance, and when not neutral they were very faintly alkaline. As Ranzi has shown that larger injections are necessary for sensitization to organ extracts than to serum, the equivalent of two grams of organ substance was used. The intoxicating dose of the various proteins was likewise the equivalent of two grams of organ substance. The sensitizing dose of dog serum, used as a control, was 0.05 cubic centimeter, and the intoxicating dose two cubic centimeters. As controls, amounts of all protein fractions and of dog serum equal

TABLE I.

The Agglutinating, Hemolyzing, and Precipitating Action of the Various Anti-Kidney Sera. Controls with Anti-Dog Blood Serum, Normal Rabbit Serum, and Physiological Salt Solution. The Uppermost Group Shows the Action of the Sera as Agglutinating Agents.

	Anti-kidney nucleoprotein serum.				Anti-kidney globulin serum.				Anti-kidney albumin serum.				Anti-blood serum.				Normal rabbit serum.				0.85 per cent. sodium chlorid solution.			
	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	24 hr.
Kidney cell suspension	+	+	+	+	+	+	+	+	—	—	—	—	+	+	+	+	+	+	+	+	—	—	—	—
Liver cell suspension	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Red blood cell suspension	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	—	+	+	+	+	—	—	—	—
Red blood cell suspension	—	—	—	—	—	—	—	—	—	—	—	+	—	trace	+	+	—	—	—	—	—	—	—	—
	½ hr.	1½ hr.	17 hr.	24 hr.	½ hr.	1½ hr.	17 hr.	24 hr.	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1½ hr.	17 hr.	24 hr.	½ hr.	1 hr.	2 hr.	24 hr.	½ hr.	1 hr.	2 hr.	24 hr.
Dog serum ($\frac{1}{10}$ dilution)	—	—	—	—	—	—	—	—	—	—	—	—	±	+	+	+	—	—	—	—	—	—	—	—
Kidney nucleoprotein solution	—	—	+	+	—	—	—	—	+	+	+	+	±	+	+	+	—	—	—	—	—	—	—	—
Kidney globulin solution	—	—	+	+	—	—	—	—	+	+	+	+	±	+	+	+	—	—	—	—	—	—	—	—
Kidney albumin solution	—	—	+	+	—	—	—	—	—	+	+	+	±	+	+	+	—	—	—	—	—	—	—	—
Liver nucleoprotein solution	—	—	+	+	—	—	—	—	—	—	+	+	±	+	+	+	—	—	—	—	—	—	—	—
Liver globulin solution	—	—	+	+	—	—	—	—	—	+	+	+	±	+	+	+	—	—	—	—	—	—	—	—
Liver albumin solution	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
0.85 per cent. sodium chlorid solution	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE II.

The Agglutinating, Hemolyzing, and Precipitating Action of the Various Anti-Liver Sera. Controls with Anti-Dog Blood Serum, Normal Rabbit Serum, and Physiological Salt Solution. The Uppermost Group Shows the Action of the Sera as Agglutinating Agents.

	Anti-liver nucleoprotein serum.				Anti-liver globulin serum.				Anti-liver albumin serum.				Anti-dog blood serum.				Normal rabbit serum.				0.85 per cent. sodium chlorid solution.			
	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	5 hr.	½ hr.	1 hr.	2 hr.	5 hr.	½ hr.	1 hr.	2 hr.	24 hr.
Kidney cell suspension	—	+	+	+	—	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—
Liver cell suspension	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Red blood cell suspension	—	—	—	—	—	—	—	—	—	—	±	±	+	+	±	—	—	—	—	—	—	—	—	—
Red blood cell suspension	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	—	—	—	—	—	—	—	—
	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	4 hr.	½ hr.	1 hr.	2 hr.	5 hr.	½ hr.	1 hr.	2 hr.	5 hr.	½ hr.	1 hr.	2 hr.	24 hr.
Dog serum ($\frac{1}{10}$ dilution)	—	+	+	+	—	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
Kidney nucleoprotein solution	—	—	—	—	—	—	+	+	—	—	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Kidney globulin solution	—	—	+	+	—	—	+	+	—	—	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Kidney albumin solution	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Liver nucleoprotein solution	—	—	—	—	±	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Liver globulin solution	—	—	±	±	±	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Liver albumin solution	—	—	+	+	—	—	+	+	—	—	+	+	—	—	—	—	—	—	—	—	—	—	—	—
0.85 per cent. sodium chlorid solution	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

to the intoxicating dose were injected intravenously without effect into normal guinea pigs.

RESULTS.

A study of table III shows that the globulins and albumins are good sensitizers and especially so for the toxic dose of protein of an homologous organ. This is shown more definitely in the liver than in the kidney series. The liver nucleoprotein fraction sensitizes to the toxic dose of liver proteins more markedly than to the kidney proteins and appears to be fully as active a sensitizer as the globulins and albumins. On the other hand, a study of the sensitizing action of the kidney nucleoprotein shows an entirely different condition, this fraction being almost inert.

The same general relation holds in reference to the toxic action of the several fractions; the globulins and albumins being distinctly more toxic than the nucleoproteins and showing also a definite predilection for the homologous organ fractions. Just as the liver nucleoprotein is more active and slightly more specific than the kidney nucleoprotein as a sensitizing agent, so it is as an intoxicating agent.

A study of the sum of the reactions shows a relative organ specificity. Eight of the nine animals sensitized to liver fractions were distinctly anaphylactic when injected with liver fractions, whereas of the nine injected with kidney protein, only two were distinctly anaphylactic, the others showing only moderate or slight symptoms. In the same way, five of the nine animals sensitized to kidney fractions were distinctly anaphylactic to kidney proteins, and a similar group of nine failed to react when injected with liver proteins. In contrast with this slight organ specificity is the fact that in no sense can any degree of protein specificity be shown.

The crossed reactions with dog serum fail to indicate specificity. When the serum is used as a sensitizer, distinct anaphylaxis was produced by the subsequent injection of organ protein fractions with one exception (liver globulin), which probably was due to some individual idiosyncrasy in the animal. As a toxic agent, the serum is active almost uniformly, the only exception being in the animals sensitized to kidney nucleoprotein, a fraction notably defi-

cient in sensitizing power throughout the entire series. Animals sensitized to dog serum react no more strongly to toxic doses of serum than do those sensitized to the various organ protein fractions.

CONCLUSIONS.

1. The sera of rabbits injected repeatedly with the nucleoproteins, globulins, and albumins of the liver and kidney of the dog give no evidence *in vitro* or *in vivo* experiments of organ affinity. The precipitin test offers no proof of the specificity of these sera for the proteins employed as antigens.

2. The anaphylaxis reaction applied to the same proteins indicates a slight relative organ affinity but no specificity as far as the respective protein fractions are concerned. The relative organ affinity resides, rather, in the globulin and albumin fractions than in the nucleoprotein fraction. Dog serum used both as a sensitizing and an intoxicating agent gives rise to very active cross reactions with organ proteins, thus failing to support the theory of organ or of protein specificity.

3. These results do not support the view put forward that nucleoproteins play an important part in the course of production of cytotoxic immune sera.

TABLE III.

Anaphylaxis.

	Toxic Doses of						
	Kidney albumin.	Kidney globulin.	Kidney nucleo-protein.	Liver albumin.	Liver globulin.	Liver nucleo-protein.	Dog serum.
Sensitized to:							
Liver albumin	M.A.	A.	M.A.	A.	A.	A.	A.
Liver globulin	S.A.	N.	A.	A.	A.	A.	A.
Liver nucleoprotein	S.A.	M.A.	S.A.	A.	A.	S.A.	A.
Kidney albumin.....	A.	A.	A.	M.A.	N.	S.A.	A.
Kidney globulin.....	A.	A.	N.	S.A.	S.A.	S.A.	A.
Kidney nucleoprotein.....	N.	N.	N.	S.A.	N.	M.A.	S.A.
Dog serum.....	A.	A.	A.	A.	M.A.	A.	A.

S.A. = slight symptoms, such as slight dyspnea, rubbing of nose, and general twitching.

M.A. = somewhat more marked symptoms, the twitching changing to slight general convulsions.

A. = very marked dyspnea, cyanosis, general convulsions, and coma, often followed by death.

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CALCIUM AND IRON INCRUSTATION AND OTHER LESIONS OF THE ELASTIC TISSUE OF THE SPLEEN AND LIVER.*

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PLATES 4 TO 7.

This report is intended as a contribution to vascular pathology and to the pathology of elastic tissue. The principal lesion described is evidently an unusual one, since a search through the literature has revealed very few similar observations, and indeed only one in connection with the spleen, namely, that reported by Marini in a case of splenomegaly, which he regarded as an example of Banti's disease. Kockel and Bittrolff have reported analogous but less extensive processes occurring in the lungs in chronic passive congestion and in pernicious anemia.

The subject from which the material to be described was obtained was autopsied at the Bay View Hospital on November 11, 1910, four hours after death. Only a meagre clinical history is available.

The patient was a white man, 59 years old, who for five months had suffered from "stomach trouble," anorexia, loss of weight, weakness, and shortness of breath. Examination showed a large protruding abdomen and marked distention of the superficial veins. The spleen was palpable over a hand's breadth below the costal margin. The abdomen was full of fluid and there was some edema of the lower extremities. The anatomical diagnosis follows.

Cholelithiasis (stone in common duct); dilatation of gall bladder and ducts; cholangeitis; cirrhosis of liver; marked dilatation of portal vessels; congestion of area supplied by portal vein with edema of intestines and mesentery; ascites; chronic splenic tumor; rupture of esophageal varices; acute and chronic pancreatitis; bronchopneumonia; arteriosclerosis, especially of smaller arteries, *e. g.*, coronaries and splenic; chronic diffuse myocarditis; congenital cystic kidneys; edema and fading purpuric eruption on legs; emaciation; slight icterus.

Only those organs of especial interest will be described further in this report.

* Received for publication, April 27, 1911.

After opening the duodenum, the bile papilla was found to be quite normal in appearance. All the bile passages were much dilated and stained a bright yellow color. Just below the juncture of the hepatic and cystic ducts, there was an oval concretion measuring 2 by 1 cm., which was freely movable and which might easily have been pushed up from a position near the ampulla during the examination. The liver was somewhat enlarged, weighing 1,700 grams; it was greenish yellow, hard, and firm, with a slightly granular surface. The cut surface showed a rather obscured lobulation with thin strands of bluish white tissue between the lobules. Opaque yellowish areas, 1 or 2 mm. in diameter, with dark red borders were seen here and there. The portal vein and its radicals were enormously dilated and somewhat tortuous.

The spleen was much enlarged, measuring 21 by 13½ by 7½ cm., and weighing 775 grams. The surface, especially of the upper pole, was covered with small, elevated, yellowish white nodules. The color of the cut section was brownish red. The pulp was diminished and the interstitial tissue correspondingly increased in amount. The consistence was somewhat increased, but there was no suggestion of the rubbery spleen of chronic passive congestion. The Malpighian bodies were small and widely spaced. Both capsule and trabeculæ were decidedly thickened and prominent and in the latter there were frequently seen small ochre-colored patches, 1 or 2 mm. in diameter, in the center of which a small vessel appeared.

HISTOLOGICAL DESCRIPTION.

Spleen.—The capsule and trabeculæ are much thickened. The outlines of the sinuses are quite distinct and their endothelial nuclei unusually prominent. The sinuses contain red blood cells and an occasional lymphocyte. In the pulp spaces there are very few cells, consisting of the usual lymphocytes and splenic pulp cells.

The striking lesion is found in those areas visible to the naked eye as ochre-colored patches. This impresses one at first as a peculiar deposit of pigment in the trabeculæ, and especially in the walls of vessels having a diameter of from one tenth to one sixth of a millimeter (figures 1 to 4). In hematoxylin and eosin preparations, the lumen of the affected vessels is usually found to be smooth and regular and lined by a thin intima. Just outside the intima there are found concentric, circular narrow bands of a glistening, golden or yellowish green color which may or may not entirely encircle the vessel. They are not absolutely continuous but appear more as broken lines or small pieces joined end to end. Beyond this zone the tissues of the trabeculæ have a very peculiar appearance. They consist of swollen bands or strands, which run sometimes around

the vessel and are sometimes quite irregular in their course, often crossing each other at various angles. They may be broken up into round or oblong masses. Some have the golden, glistening appearance already noted, others take a deep hematoxylin stain, either homogeneously or in clumps of granules here and there. They go over rather abruptly into the more normal appearing trabecular tissue. Between the broader bands and masses a delicate blue-staining fibrillar network may occasionally be demonstrated. The lesion is found not only in the vessels of the trabeculæ, but also in many of the arteries within the splenic pulp and often at a point just before they divide into the smaller intralobular branches.

The vessels, which are only partially affected, are particularly instructive, for here it is seen that the elastic tissue is especially concerned. The internal elastic lamella may be traced from the normal wavy, refractile membrane into a less wavy, blue-staining fiber, which goes over directly into the rigid, broken, impregnated structure already described (figure 5). The same transition may be traced in other elastic fibers of the vessel wall, the outer fibers often appearing broken and impregnated, while the immediately adjacent internal lamella is relatively normal. In other places, where the lesion is apparently recent, a vessel is surrounded by two or three concentric bands, and in the trabecula many hematoxylin-staining fibers appear, more or less wavy and a little broader than the refractile elastic fibers with which they are continuous. In addition, there is seen here and there a blue-staining fibrillar network and many connective tissue cells filled with a golden, granular pigment.

While, as a rule, the bands or fibers near the lumen of the vessel appear yellowish green and refractile and those further removed stain with hematoxylin, this is by no means invariable. The inner layers may be homogeneously blue and the outer heavy bands in places possess the glistening greenish characteristics. Either one may be present exclusively. In unstained sections, the impregnated tissues are conspicuous and of a beautiful golden color, which is quite homogeneous in some of the bands, while in others it occurs in globules or even in clumps and strings of small granules. Be-

tween and around the heavier bands, a delicate golden network suggests the elastic fibrils.

MICROCHEMISTRY.

The affected tissues give a pronounced reaction for iron with ammonium sulphid (figure 6), potassium ferrocyanid and hydrochloric acid, and with aqueous hematoxylin. With the ferricyanid of potassium, only a very weak reaction is obtained.

The impregnating salts are readily dissolved by dilute mineral acids without the formation of gas bubbles. If sulphuric acid is used, there is a deposit of the needle-shaped crystals of calcium sulphate, and after dissolving out the iron in half-saturated aqueous solution of oxalic acid, the calcium salts may be demonstrated by the violet stain with aqueous hematoxylin. This is found especially in those areas which with hematoxylin and eosin take a deep hematoxylin stain. After prolonged treatment with oxalic acid, crystals of calcium oxalate are deposited.

Phosphates are easily shown by treating sections with a 3 per cent. solution of silver nitrate. After exposure to light, the encrusted tissues become black. If 1.5 per cent. of nitric acid is added to the silver nitrate solution in the attempt to differentiate chlorids, which are insoluble in dilute nitric acid while the phosphates are soluble, the reaction is no longer obtained. A certain amount of pigment in the peripheral parts of the trabeculæ remain unchanged. By using the method for demonstrating phosphates recommended by Macallum, the exposure of sections to a solution of ammonium molybdate in nitric acid and subsequent treatment in a dilute solution of phenylhydrazin-hydrochlorid, the reaction is positive throughout most of the impregnated tissues, which become greenish blue in color. The bands which in other preparations did not take the hematoxylin stain behave somewhat differently. Almost immediately after immersion in the first fluid, the glistening golden material seems to be partially dissolved and then deposited in small irregularly round masses slightly larger than red blood cells, which, transmitting very little light, appear almost black. Those which are broken up seem to consist of small yellow granules quite similar to the original material. After treatment with phenylhydrazin, they

become bluish in color. Much of the golden material, however, is not so changed and remains in its original position and of the same appearance. So far as may be judged, then, by these reactions, the metals, calcium and iron, are present chiefly as phosphates.

ELASTIC TISSUE.

In studying the elastic tissue, Weigert's, Verhoeff's, and Unna's orcein methods were used. By first treating sections with potassium ferrocyanid and hydrochloric acid and then staining with orcein, the iron-containing tissues are colored blue and the elastic tissue becomes a silky brown. By this method the relations between the two are best studied, especially in the less advanced lesions. Here the brown elastic fibers are directly continuous with the blue, iron-containing tissues, some of which preserve something of the characteristic morphology of elastic fibers. If Perl's stain is not used, the acid content of the Weigert and orcein stains dissolves the salts from the petrified tissues, which then become prominent by reason of their lack of elastic staining. The innermost layer of the internal elastica usually retains the stain, appearing stiff and much thickened without a trace of its usual wavings, and occasionally one of the thick bands in the trabeculæ close by, colors intensely with the elective stain. Small elastin granules are sometimes numerous. In hematoxylin and eosin preparations, the demineralised tissues have a hyaline appearance.

With Grübler's polychrome methylene-blue, the incrusted tissues retain a deep blue stain after all the other tissues are decolorised, thus giving the so-called "elacin" reaction of Unna. The reaction is negative in sections previously treated with acid.

The collagenous and muscular tissues must be affected in the advanced lesion, but from a study of the earlier stages it seems clear that the elastic tissue is primarily concerned.

The elastic tissue of the thickened capsule and trabeculæ generally throughout the sections is not only absolutely but also relatively increased. The network is denser, each fiber being larger and staining more intensely than in the normal spleen. About the intralobular arteries there appears very frequently a pronounced net-

work, which is also sometimes conspicuous about the periphery of a Malpighian corpuscle.

Retrogressive changes in the elastic tissue, other than the swelling and incrustation of its fibers, may be noted. The internal elastic lamella of a vessel is frequently represented by two distinct, thick, wavy lines, but in this case it is often divided into four or five parallel wavy strands. In a few instances in the larger vessels, it is split up into eight or ten fibrils which form a spindle in the course of the elastica. Into this spindle the fibrils run, become straight, more and more beaded, and finally break up into small granules which retain the elective stains. Such a spindle may be a third of a millimeter in length (figure 7). Somewhat similar appearances are described by Mönckeberg in the internal elastica of peripheral arteries over calcified areas in the media.

Again, in certain trabeculæ it is found that the dense, deeply staining network goes over rather suddenly into an area in which the fibrils are delicate, lightly staining, widely spaced, straighter, and arranged in a less definite network. With the combined Perl's and orcein stain many of these fibrils show blue masses adhering to them or incrusting them, and for a short distance the fibril may stain blue instead of brown. This finding is more frequent and more marked in such atrophic areas than in the more nearly normal trabeculæ.

The Malpighian corpuscles are far apart and most of them are normal. Near the center of some of them, the lymphoid cells seem forced apart by a hyaline material staining pink with eosin, which may occur in small, refractile globules or be fused into larger areas half a millimeter in diameter, in which are embedded here and there nuclei of lymphoid cells. With Perl's and orcein stains, a few small, beaded, elastic fibers are seen in direct connection with these areas, which stain, however, a little less intensely with the orcein. Irregularly round, iron-containing bodies about six times as large as a lymphoid cell are occasionally found in this hyaline substance (figure 8).

Liver.—The cirrhosis is an early and, therefore, interesting example of the type usually known as biliary cirrhosis. The bile ducts are dilated and often filled with polymorphonuclear leucocytes,

which may extend into the surrounding tissues. The new connective tissue is cellular and principally localized in the periportal areas, from which fibroblasts may be seen growing in between the strands of liver cells. In addition, there is a recent, extensive, central necrosis in many, but not in all, of the lobules. In the denser connective tissue strands there are occasionally seen small, oblong or rectangular structures which stain intensely with hematoxylin. Some of these appear as dark blue, wavy fibers (figure 9).

The elastic tissue is present in increased amounts in the connective tissue strands, but the fibrils are often broken into fine granules. Apparently the same small, oblong or wavy structures stain intensely brown with orcein. In unstained sections, they appear yellowish and refractile. In sections treated with silver nitrate, they become black after exposure to light. These calcified elastic fibers in the liver do not give an iron reaction.

The selective impregnation of the elastic fibers with mineral salts is in striking contrast to the calcification usually observed in the walls of vessels.

Mönckeberg states that in calcification of the media in peripheral arteries the elastic tissue is the last constituent of the media to be affected. Klotz emphasizes the immunity of elastic fibers in primary medial calcification of arteries of the elastic tissue type. The lesion here described is not confined to the vessels but affects the tissues of the splenic trabeculæ to even a greater extent, and, therefore, may not strictly be compared to the usual forms of vascular disease.

Marini describes a similar but much less extensive change in the elastic tissue of a very large spleen which weighed 1,900 grams. There was also an atrophic cirrhosis of the liver. The principal lesions in the spleen were in the Malpighian corpuscles, which were sclerotic from a new growth of both collagenous and elastic fibers. In the arterioles of the follicles in many places, he says, the intima, media, and adventitia had disappeared and were replaced by pieces of a substance which in hematoxylin and eosin preparations appeared yellowish and refractile to light. Tests for calcium and for carbonates were negative. The substance was present only in places from which the elastic tissue had disappeared; it gave a pronounced iron reaction as well as the reaction for the degenerated material of elastic tissue, which Unna designated as "elacin." Both of these reactions disappeared after treating the sections with acid. Marini thought that the process might be explained by the chemical transformation of elastin into elacin, and that the iron might have been derived from some iron content of the elastin from which the elacin was derived, or that it was due to blood pigment.

Kockel and, recently, Bittrolff have each described a case of calcium and iron incrustation of the elastic fibers in lungs which were the seat of chronic passive congestion. The lesion was present in the walls of small vessels and in the

elastic tissue of the alveolar walls. It was more extensive in Bittrolff's case, and the appearances described and pictured by him are in many respects quite similar to those reported in this paper. In using Perl's and orcein combination stain, he found that some of the elastic fibers colored brown in part of their course, blue in other parts, and a mixed color in the transitions from the one into the other. He aptly compares the appearance of the broken incrusted elastic fibers to that of chains of anthrax bacilli. A striking feature of his case was the presence of many large, multinucleated giant cells of the foreign body type, each containing broken fragments of the degenerated elastic fibers. Giant cells were present in Kockel's case, but not in such great numbers. Kockel examined a series of lungs and found similar lesions of very slight degree in three with chronic passive congestion.

There are several other observations in the literature of calcification of elastic fibers, and in some of them iron was also demonstrated. Jores, Ernst, and Matusewicz describe isolated calcification of the membrana elastica interna. Jores considers this of frequent occurrence in the vessels of goitres and has observed it in arteriosclerosis, although in most cases of sclerosis the internal elastic lamella is well preserved.

Rona and Hektoen report calcified and iron-containing elastic fibers in giant cells in various inflammatory processes. In examining a series of cases of lupus, Rona found this lesion in three fourths of the cases. Since he saw no calcified fibers outside of the giant cells, he came to the conclusion that the calcification had occurred within the cells, that the slower metabolism within the giant cells probably accounted for the persistence of the elastic elements enclosed within them, while those outside were early destroyed by the bacterial poisons. In several of his cases iron was also demonstrable in the calcified fibers and usually in those lesions from the lower extremities. He thinks that chronic passive congestion and hemorrhagic inflammations are of importance in determining its presence.

S. Ehrlich reports several instances of simultaneous calcium and iron incrustation of elastic fibers in scars of splenic infarctions, in an intestinal polyp, in the alveolar walls of the lungs, and in other lesions. He says his observations show that elastic fibers in the neighborhood of hemorrhages have a special tendency to absorb iron and that such fibers may secondarily become the seat of calcium deposits. He agrees with Schmorl that the iron deposit precedes that of the calcium for which it acts as a kind of mordant.

Gierke found a positive iron reaction not only in many examples of pathological calcification but also in certain physiological calcium deposits, notably in fetal bones, and in the embryonic enamel and dentine of guinea pigs.

Hueck, however, protests against the interpretation that such reactions indicate that iron occurs along with calcium during life, and he insists that most of the reported findings are artifacts due to the absorption of iron by the calcified tissues from the fluids in which the tissue is fixed or preserved. He points out several sources of error, stating that the reagents obtained from the best manufacturers are not always iron-free and that, if the tissue contains blood pigment, this may be dissolved by the fluids used and subsequently be absorbed by the calcified areas. He bases his arguments upon his own investigations of fresh material with negative results and upon a rather convincing experiment. He

examined the tissues from young mice in two parallel series, in one of which the sections were cut in paraffin and in the other in celloidin. In the paraffin sections, which had remained in the fluids only a few days, there was no iron. The celloidin sections were exposed to the reagents longer and showed an intense iron reaction only in the calcified areas. On examining the absolute alcohol used for the celloidin tissues, he found that it contained iron.

Hueck's criticisms apply with most force to those reports of positive iron reactions occurring in physiologically calcified tissues. Bittrolff remarks that there seems no good reason why the calcified, presumably necrotic, elastic fibers in his case might not absorb iron during the life of the patient from the fluids rich in iron, in which they were constantly bathed, if calcified tissues show such a predilection for iron after death. It may be added here that in this case the ochre color of the lesion at autopsy, a few hours after death, and the fact that tissue fixed at once in formalin and examined the next day showed as much iron as that examined several months later, indicate clearly that in such lesions the iron and calcium are coëxistent during the life of the subject.

It is difficult to determine which process is primary. The apparent predominance of the iron in this case and the occurrence of iron without calcium in some isolated fibers of the splenic trabeculæ might lead one to consider the iron of prime importance. Ehrlich, too, claims that iron incrustation may occur independently of calcification. On the other hand, we know that calcified tissues in general are usually free from iron or show small traces of it. In this case, we have calcified elastic fibers in the liver, but no iron. Practically all the reported observations of iron incrustation of elastic fibers have been in organs or tissues showing either hemorrhages or marked congestion; Kockel's and Bittrolff's in congested lungs, Marini's and my case in spleens with cirrhotic livers, Hektoen's in a hemorrhoidal nodule, Ehrlich's in a hemorrhagic rectal polyp, splenic infarction, etc. Rona's explanation, already mentioned, for the iron deposited in giant cells in lupus is the same. It seems probable, then, that iron is absorbed secondarily by the calcium deposits when the surrounding tissues are rich in that element in inorganic combination, but Marini's and some of Ehrlich's observations do not permit us to exclude the possibility that iron may incrust elastic fibers without the mediation of calcium.[1]

[1] Since the preparation of this paper, there has come to the author's attention an article by Sumita, "Zur Frage des Eisenreaction kalkhaltiger Gewebe, insbesondere des Knochens," *Virchows Arch. f. path. Anat.*, 1910, cc, 220. On the basis of careful experiments, Sumita concludes that the deposit of iron precedes that of calcium not only under pathological conditions but also in the physiological formation of bone.

If we assume the primary character of the calcium deposits, the reason for their occurrence is quite as obscure as the cause of calcification in general. It is a generally accepted principle in pathology that only extremely degenerated or necrobiotic tissue becomes the seat of pathological calcium deposits. In the apparently advancing margins of this lesion, however, it was not possible to demonstrate by the methods used any abnormality of the elastic tissue until after the deposit of the salts. Chemical change in the petrified fibers is shown by their failure to take the selective elastic stains and by the deep blue stain with methylene-blue in alkaline solution, which does not affect the normal elastic tissue. This degenerated substance Unna speaks of as elacin, in contradistinction to the elastin of the normal tissue. This reaction disappeared simultaneously with the iron reaction after treating sections with acids, but it can not depend entirely upon the presence of inorganic iron, since this substance, under other conditions, in tissues near hemorrhages, in hemochromatosis, etc., does not give the reaction. The intracellular iron pigment in the same sections fails to take the blue stain. Ordinary calcified areas, too, lose the stain when the nuclei are decolorized. In view of the work of Klotz on calcification, a careful search was made for any fatty substances which might be present, but none were demonstrated. Frozen sections of formalin-fixed tissues were stained in Sudan III for twenty-four hours, both before and after dissolving out the salts in different acids.

A puzzling feature of these cases is the occurrence in some of them of great numbers of giant cells, and of their entire absence in others. Kockel, Hektoen, and Bittrolff speak of them as phagocytic for the degenerated elastic elements. Rona and Unna do not accept any such activity for the giant cells in lupus, and they think the fibers which chance to be enclosed within the cells are protected to a certain extent from the injurious agents which cause the rapid degeneration of those outside the cells. No giant cells were seen in the neighborhood of the incrusted fibers in this case, nor were there any other appearances indicative of a foreign body reaction.

The hyperplasia of elastic tissue in the spleen and liver is in harmony with the results reported by Melnikow-Raswedenkow, who investigated the elastic tissue of a number of organs under normal

and pathological conditions. The greatest hyperplasia in the spleen was found in chronic splenic tumors occurring as a result of cirrhosis of the liver. An elastic tissue increase was the rule also in cirrhotic livers. The author referred the growth of elastic tissue to mechanical causes; increased pressure due to congestion, for example. It is possible that in this case the condition of strain resulting from the early stages of congestion was responsible for the hyperplasia of the elastic elements and later, passing into a condition of stress, brought about the regressive changes, which resulted in the calcium and iron incrustation. In this connection the great dilatation and slight sclerosis of the portal and splenic veins should be remembered.

The effect of tension upon degenerative changes of elastic tissue has been studied experimentally by Harvey. He buried guinea pigs' aortas, both collapsed and distended under pressure, within the tissues of other animals of the same species and examined the tissues at different time intervals. He found that the elastic fibers were much more resistant than other portions of the vessel wall and that this power of resistance was much lowered when the tissue was placed under tension. The collapsed aorta, examined after seventy-five days, showed beginning calcification of some of the heavier elastic fibers, and after 106 days there was extensive calcification. The aortas buried under tension showed complete disappearance of the elastic tissue after fifty days. Those examined after shorter intervals showed no calcification.

The lesion in Marini's case might be explained as a result of the action of the hypothetical toxin which, according to Banti, is responsible for all the features of the disease that goes by his name, including the splenic enlargement, anemia, sclerosis of the splenic and portal veins, and cirrhosis of the liver. It seems possible, if not altogether probable, that the case of this report may also be an example of primary splenomegaly and that the splenic lesions are explicable on this basis. The size and gross appearance of the spleen, the histological picture in general, and the lesions in the Malpighian corpuscles, all support this view. The cirrhosis of the liver is not of the type secondary to splenic enlargement, but seems definitely due to the infectious process in the bile passages, with an

added terminal central necrosis. However, the cirrhosis seems to be of quite recent origin, the lobulation is well preserved, and the newly formed connective tissue is limited to the periportal areas and is quite cellular. The relatively advanced changes in the spleen seem to be out of all proportion to the grade of cirrhosis in the liver. On the other hand, there was definite portal obstruction with ascites, marked esophageal varices, and dilatation of the superficial abdominal veins. In the absence of a clinical history, we must leave it an open question, whether we have a primary splenomegaly with a superadded biliary cirrhosis or whether the whole condition is the result of the hepatic disease.

For reviews of the subjects of progressive and regressive changes of elastic tissue, the reader is referred to the papers by Jores. I have seen no other reported observation of calcified elastic fibers in the cirrhotic liver.

SUMMARY.

In conclusion, it may be said that the lesion described consists of a selective impregnation of the elastic tissue in the splenic trabeculæ and vessels with the phosphates—and perhaps other salts—of calcium and iron. There is a further chemical change in the elastic fibers which may be expressed by the term "elacin."

Degenerated and, especially, calcified elastic tissue—even more than calcification in general—seems to possess an affinity for iron salts. The iron incrustations occur principally in congested or hemorrhagic tissues.

The etiological agent in this case is obscure. The only other example of this lesion in the spleen was in a case of Banti's disease. Many features of this case suggest a primary splenomegaly, but the evidence in its favor is not conclusive.

Calcified elastic fibers, without the iron and elacin reactions, were also present in the liver, which was the seat of a recent, apparently biliary or infectious, cirrhosis. The elastic tissue of other organs showed nothing remarkable except in the skin, where the fibers were undergoing the usual senile changes.

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EXPLANATION OF PLATES.

Plates 4 and 5.

Figs. 1 to 5. Stained with hematoxylin and eosin. Different stages of the process in the splenic vessels and trabeculæ. The bands about the vessels in

figures 2 and 4 and about the smaller vessel in figure 1 were refractile and yellowish green. The rest took the hematoxylin stain in different degrees of intensity. Note in figure 3 the fine blue fibrils above and to the right of the vessel and between the heavier bands.

PLATE 6.

FIG. 5. Different degrees of impregnation of the elastica interna.

FIG. 6. Splenic vessel and trabecula, showing marked iron reaction with ammonium sulphid.

FIG. 7. Spindle-shaped area of fibrillation and granulation in the course of the elastica interna of a larger splenic artery, about one millimeter in diameter. Orcein stain.

PLATE 7.

FIG. 8. High power of center of Malpighian corpuscle showing the hyaline areas. Orcein combined with Perl's stain. Note the beaded elastic fibers running from the lower left corner toward the center. The round, dark bodies —two of them indicated by arrows—are iron-containing, staining blue.

FIG. 9. Liver. Note the dark, wavy, calcified elastic fibers. Hematoxylin and eosin.

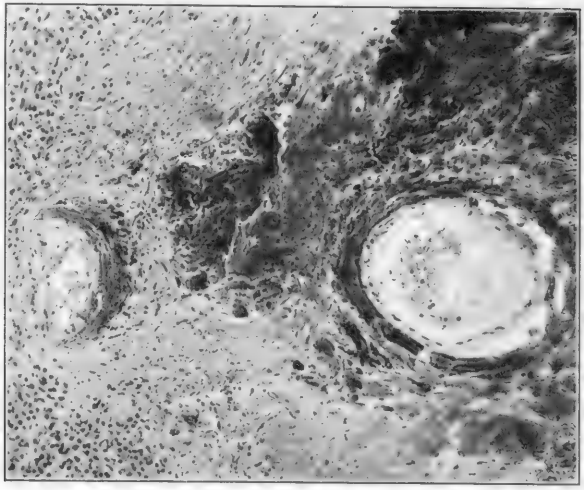

FIG. 1.

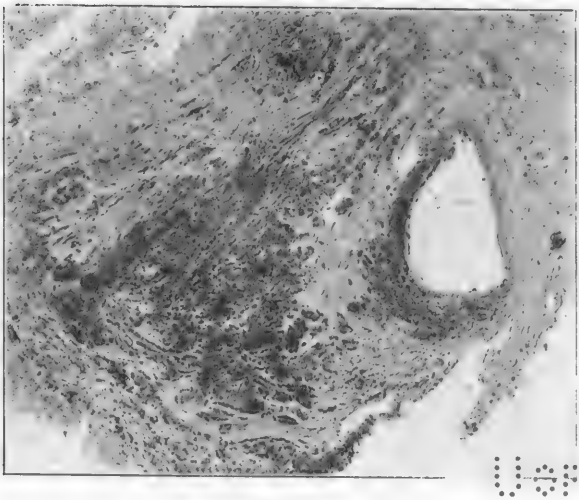

FIG. 2.

FIG. 3.

FIG. 4.

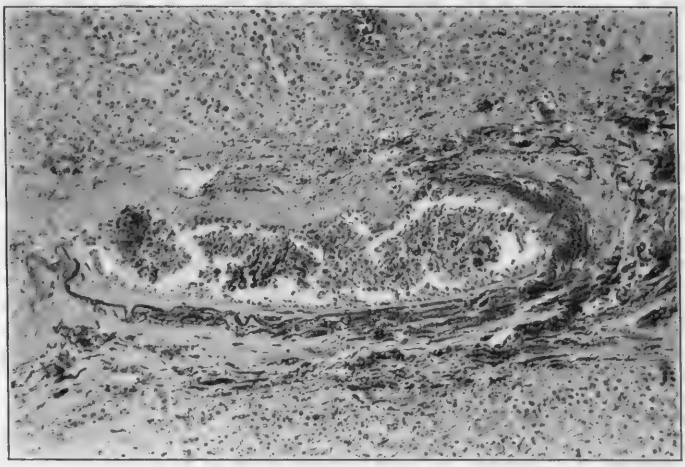

FIG. 5.

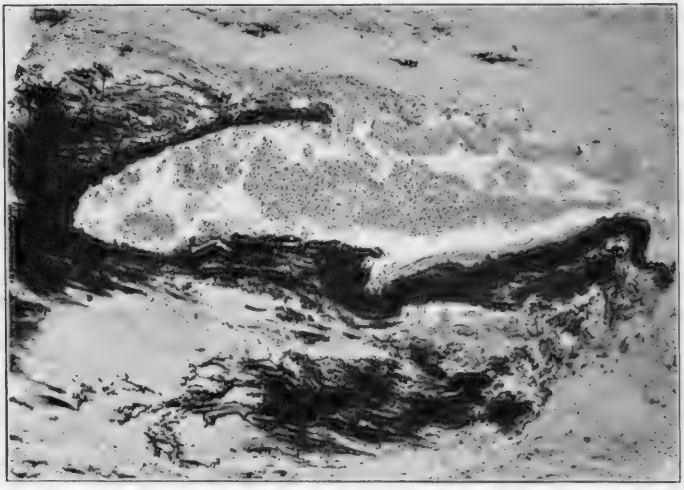

FIG. 6

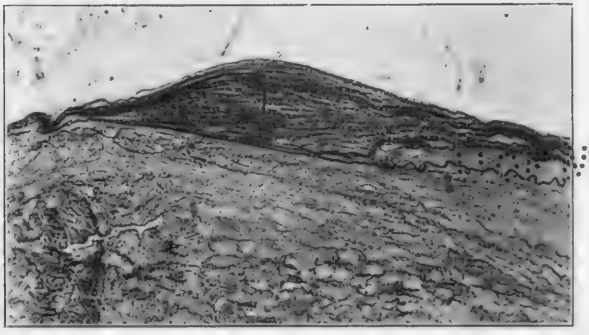

FIG. 7.

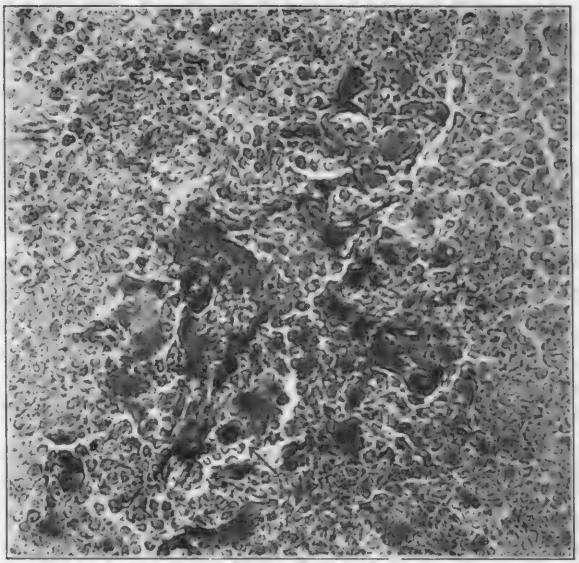

FIG. 8.

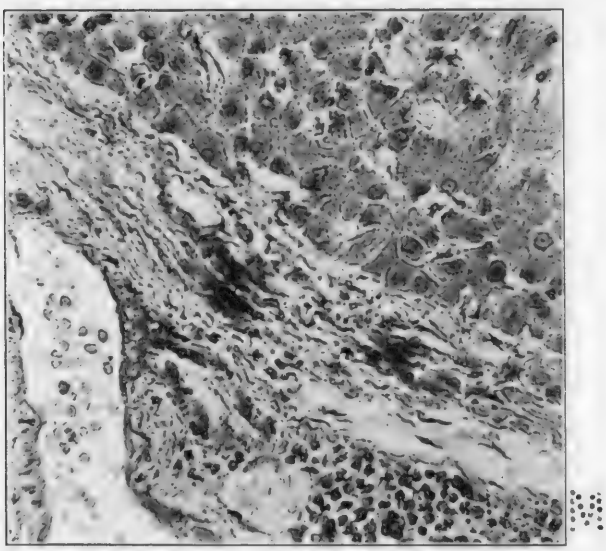

FIG. 9.

SERUM TREATMENT OF INFLUENZAL MENINGITIS.*

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Our precise knowledge of the bacteriological causes of acute cerebrospinal meningitis has been greatly promoted by the employment of lumbar puncture as an aid to the diagnosis and to the treatment of that condition. We are now in a position to define quite accurately the several bacterial forms of acute meningitis and to state their relative frequency and degree of fatality. Among the highly important gains in knowledge of acute meningitis is the fact that the influenza bacillus is by no means an infrequent cause of that condition, and, further, that influenzal meningitis has terminated fatally in all but six of the fifty-eight pure cases thus far reported. The number of reported cases of influenzal meningitis is increasing rapidly, probably because their frequency and importance are becoming more impressive as the bacteriological diagnosis of cerebrospinal meningitis is becoming more widely known and more commonly performed.[1]

The frequency and severity of influenzal meningitis were impressed upon us at the Rockefeller Institute, where, for a period of several years, large numbers of specimens of cerebrospinal fluids have been examined bacteriologically. These fluids were sent for examination on the supposition that they were obtained from cases of epidemic meningitis, so the diagnosis was unexpected. All the cases diagnosed by us as influenzal meningitis terminated fatally.

The impulse to study influenzal meningitis experimentally came in part from the circumstance of its high fatality, and in part

* Received for publication, May 18, 1911.

[1] Wollstein, Influenzal Meningitis and Its Experimental Production, *Am. Jour. Dis. Child.*, 1911, i, 42; Dunn, Cerebrospinal Meningitis, Its Etiology, Diagnosis, Prognosis and Treatment, *ibid.*, 85; Davis, Influenzal Meningitis, with Especial Reference to Its Pathology and Bacteriology, *ibid.*, 249.

constituted a feature of a more general investigation that has been conducted, under Dr. Flexner's supervision, for several years, on the control of localized infectious processes within the body. The first step in this experimental study was, obviously, the production experimentally of a condition closely resembling influenzal meningitis in man.

First, a word as to influenzal meningitis in human beings. The disease is more frequent among infants and children than among adults. It sometimes follows upon undoubted influenzal disease of the respiratory tract, and sometimes develops independently of obvious disease of that tract. Since the influenza bacillus is often present in the secretions of the respiratory mucous membrane in children suffering from a variety of diseases, during the wide prevalence of influenza, it is probable that the infection of the meninges is always secondary to respiratory infection and is accomplished chiefly through the blood current. Direct infection from the nose cannot be excluded and should be considered as a possibility. The probability of an ascending infection of the meninges from the nasal mucosa is supported by the observation that in monkeys the influenza bacillus appears in the nasal mucus after being injected into the subdural space of the spinal canal by lumbar puncture. However, all or nearly all cases of spontaneous influenzal meningitis in human beings are examples of influenzal bacteremia, since the bacilli have been cultivated in large numbers, before and after death, from the heart's blood. The same general fact is true of experimental influenzal meningitis in the monkey. When the condition is produced by means of a subdural injection of a virulent culture, the bacilli can be cultivated during life and at autopsy from the heart's blood.

We have been successful, as was stated briefly in a former publication,[2] in producing a condition of acute meningitis in monkeys that resembles the spontaneous disease occurring in human beings in respect to the clinical course and symptoms and the pathological effects. But not all strains of influenza bacilli suffice to set up a severe and fatal meningitis in monkeys when cultures are injected into the subdural space by lumbar puncture. The conditions under which virulent strains have been secured are themselves important

[2] Wollstein, *loc. cit.*

and informing in respect to the significance of the influenzal bacillus infections in human beings.

EXPERIMENTAL INFLUENZAL MENINGITIS.

The first step in the experimental work is the finding of a virulent strain of *Bacillus influenzæ*. The selections are first made by tests upon white mice, guinea pigs, and rabbits. The bacilli which are isolated from the respiratory organs during life or after death are, as a rule, virulent for mice and guinea pigs. Of sixty strains so tested, only three were devoid of all virulence. The case is quite different for the rabbit, since only one strain derived from the lungs showed virulence, and in the human patient from which it was obtained, a blood invasion had taken place.

To be contrasted with this finding is the next one, namely, that all the meningeal strains except one (six in number) tested upon young rabbits, proved to be virulent. The exceptional strain was inoculated into a green monkey (*Cercopithecus callitrichus*) by lumbar puncture, and was found to be non-virulent. This result indicates in a general way, that the rabbit and the monkey react similarly to non-virulent strains of *Bacillus influenzæ*. The correspondence is, however, not exact. We have noted that a strain of *Bacillus influenzæ*, derived from a case of human meningitis, which was at first pathogenic for rabbits and monkeys, might after frequent transplantation, remain actively virulent for the rabbit and yet lose a considerable part of its power to infect monkeys. It also happens that a virulent strain may lose, after a long series of transfers outside the body, all pathogenicity for rabbits and monkeys.

The successful production of influenzal meningitis in the monkey depends upon the selection of a virulent culture and the maintenance of the pathogenicity. We have endeavored to maintain the virulence by frequent passage through the rabbit and occasional passage through the monkey. Such pathogenic cultures are grown upon blood agar slants for twenty-four hours, when they are washed down with salt solution. The effective dose consists of two cultures suspended in one or two cubic centimeters of this fluid and injected into the spinal canal in the lumbar region.

The effects of the subdural injection begin to be apparent in about five hours after the inoculation. The first noticeable effect is a disinclination to move about actively, although the monkey may not show severe symptoms for twelve or even twenty-four hours. Death may result as early as thirty-six hours after the inoculation, or it may be delayed for three or four days. A characteristic example of the longer period is as follows:—

Experiment IV.—A monkey (*Macacus rhesus*) received two slant cultures of *B. influenzæ* subdurally in the lumbar region. No immediate effect. Five hours later the monkey was unusually quiet and disposed to sit on the bottom of the cage. On being disturbed it moved about actively. After twenty hours it was still quiet and less disposed to be active. No food taken. Lumbar puncture yielded a turbid fluid containing many polymorphonuclear leucocytes and influenza bacilli. Almost all the extracellular bacilli grew profusely in culture. The symptoms grew progressively severer during the day. On the second day, the animal lay curled up, refused food, but noticed surrounding objects. Lumbar puncture yielded a fluid more turbid than before, containing an increased number of bacilli and many leucocytes. A degree of phagocytosis occurred. Cultures grew profusely. The symptoms progressed during the next twenty-four hours and the fluid obtained by lumbar puncture had become highly purulent. Death took place on the fourth day after inoculation.

Autopsy.—The brain showed purulent exudate over the cortex of the hemispheres, and the cord showed a similar exudate in the lumbar region. The pia arachnoid was everywhere cloudy. Pure cultures of *B. influenzæ* were obtained from the brain and cord. Outside the region of the central nervous system two foci of influenzal infection occurred; a lobular pneumonia of the lower lobe of the right lung over which the pleura showed a fibrino-purulent exudate, and a fibrino-purulent exudate over the anterior border of the right lobe of the liver. The influenza bacillus was present in pure culture. Sections prepared from the brain and cord showed a fibrino-purulent inflammation limited to the pia arachnoid. The heart's blood contained the bacilli.

The monkey that died thirty-six hours after inoculation showed inflammation of the pia arachnoid of the brain and cord, but no secondary foci of infection. Cultures from all parts of the central nervous system and from the upper nasal mucosa gave *B. influenzæ*.

THERAPEUTIC EXPERIMENTS.

That an acutely fatal form of influenzal meningitis can be produced experimentally in monkeys had now been established. The establishment of this fact was essential to the next step; namely, the attempt to control the condition therapeutically. With this purpose in mind, we had begun some months earlier to immunize a goat with the influenza bacillus, so that an immune goat serum was avail-

able for the experiments. Our plans involved two lines of procedure: (1) to determine the value of the immune serum as such, and (2) to attempt to intensify its activity by the addition of bactericidal chemical agents. Of the latter we had in mind, in view of Lamar's[3] studies on the pneumococcus, the use of certain bactericidal soaps. Preliminary experiments quickly showed, however, that oleate soaps in strengths below 1 per cent. exerted little effect on cultures of *Bacillus influenzæ* and, also, that the soap and immune serum mixtures were of no avail in restraining the progress of experimental influenzal infections in the monkey, but tended rather to hasten the development of the meningitis when injected into the subdural space, by reducing the local resistance of the tissues. There remained, therefore, the immune serum alone to be tried.

Throughout this study we have had in mind the treatment of the infection by local application of the therapeutic agent. The convincing demonstration of the value of the antimeningitis serum when applied in this manner and its want of all action when introduced into the blood, directed our efforts.[4] It is a very simple matter to bring the serum into the cerebrospinal membranes by direct injection, and quite impossible to cause it to be secreted there from the blood.

In influenzal meningitis, whether spontaneously acquired in human beings or produced experimentally in animals, we have to deal not only with an extensive local infection and inflammation about the sensitive nervous organs, but with a bacteremia as well. The question therefore arose, whether the control of the local infection could be effective in view of the general infection. We have viewed this question as of subordinate importance, on the supposition that the invasion of the blood by the bacilli was produced by their local development in the meninges, the restraint of which would be followed by their disappearance from the circulation. That this view

[3] Lamar, Chemo-Immunological Studies on Localized Infections. Lysis of the Pneumococcus and Hemolysis by Certain Fatty Acids and Their Alkali Soaps, *Jour. Exper. Med.*, 1911, xiii, 380.

[4] Flexner, Concerning a Serum-Therapy for Experimental Infection with *Diplococcus Intracellularis*, *Jour. Exper. Med.*, 1907, ix, 168; Flexner and Jobling, An Analysis of Four Hundred Cases of Epidemic Meningitis Treated with the Antimeningitis Serum, *Jour. Exper. Med.*, 1908, x, 690.

is well founded, the results of the therapeutic experiments proved. On the other hand, there can be no real objection to the introduction of serum into the blood either by subcutaneous or direct intravenous injection, in order to assist in the control of the bacteremia. While it has not been necessary to resort to this measure in the monkeys, it might be desirable to do so in the case of human beings.

The goat that supplied the immune serum was injected at intervals with living cultures of *Bacillus influenzæ,* for a period of about eighteen months. We determined that normal goat serum was devoid of agglutinin and opsonin for the influenza bacillus. The first cultures employed came from the respiratory tract of children, were non-virulent for animals, and produced no reaction in the goat. After two months, the blood reactions had not changed. At this time, a virulent strain of the bacillus obtained from the respiratory tract became available for injection. A reaction was immediately set up, and agglutinins and opsonins began to appear in the blood. After eight months' treatment, the agglutinins measured 1 to 80 and the opsonins 1 to 2,000. The first therapeutic test was made with the serum taken at this time (experiment V). At the end of eighteen months' treatment, the opsonic value had risen to 1 to 5,000. The serum is not bactericidal *in vitro* and does not give rise to complement fixation in dilutions greater than 1 to 100. The later experiments with monkeys were all made with the serum taken from the goat after one and one half year's immunization.

Experiment V supplied the indication that an immune serum might, under favorable conditions, control an experimental influenza bacillus infection of the meninges in the monkey. The fact is first to be observed that the interval elapsing between the infection and beginning of treatment was only one hour, and second, that the animal passed through a severe illness from which recovery appeared to follow, but that in spite of the abolition of the meningeal infection, the animal succumbed to an influenzal lobular pneumonia attended by empyema.

Experiment V.—A monkey (*Macacus rhesus*) received by lumbar puncture, two slant cultures of *B. influenzæ,* and one hour later 2 c.c. of immune goat's serum. Twenty-four hours later the animal was very ill. Lumbar puncture yielded turbid fluid containing many bacilli; culture pure and abundant. Two

cubic centimeters of serum injected. During the next twenty-four hours the symptoms were abating. Lumbar puncture gave turbid fluid still containing bacilli but showing increased phagocytosis. A third injection of the serum was given, which was followed by rapid improvement and what appeared to be final recovery. The third specimen of cerebrospinal fluid was nearly clear and contained few bacilli, which, however, still grew in cultures. A clear sterile fluid was secured on the fourteenth day. Death occurred on the twenty-fourth day. The membranes about the brain and spinal cord were normal; the lungs showed lobular foci of pneumonia and pus in the pleural cavities. Cultures from the central nervous system were sterile; but the pleural pus yielded *B. influenzæ.*

The experiments were unavoidably interrupted at this period, to be resumed some months later. The next series was carried out with a virulent culture of *Bacillus influenzæ* obtained from the meninges of a fatal case of influenzal meningitis, and with the immune goat's serum after eighteen months of treatment. Two slant cultures injected subdurally in the control monkey produced meningitis with the attending characteristic symptoms and effects that ended fatally in three and one half days.

Experiment VI.—Macacus rhesus. Usual inoculation. First serum injection one hour later. Animal ill after twenty-four hours. After second serum injection improvement began. Three serum injections given. From the third day on, the animal was lively. The animal survived. The spinal fluid one day after inoculation was turbid and contained many bacilli and leucocytes; little phagocytosis. The next day the fluid was turbid, but phagocytosis was more pronounced; very few colonies developed in culture. On the third day, the spinal fluid was nearly clear and no bacilli were found in films; two colonies grew in culture.

This experiment, although far from being a severe test, was much more successful than the similar one made with the goat's serum nine months earlier. The striking difference is seen in the rapidity with which, under the influence of the serum injections, the cerebrospinal fluid had lost its turbidity; the multiplications of the bacilli had been arrested—probably through the promotion of phagocytosis—and cultures were sterile.

Experiment VII.—Usual inoculation. First serum injection of 2.5 c.c. three hours later. At this injection the spinal fluid was already turbid from bacilli and leucocytes. The next day, the animal was very ill. Second serum injection given. Specimen of spinal fluid removed showed many bacilli and leucocytes, the former almost entirely within the cells. Third day, animal still ill; 2.5 c.c. serum injected. Fluid removed was turbid and showed leucocytes,

many of which contained bacilli; no free bacilli seen. Culture positive. Fourth day, animal recovering; 1 c.c. serum injected. Fluid still somewhat turbid; few leucocytes containing bacilli; no free organisms; slight growth in culture; Fifth day, fluid clear; no bacilli; culture negative. Recovery complete.

This animal was desperately ill on the second day and it seemed hardly possible that recovery could be secured. The course of the disease resembled that often seen in human beings, and yet terminated favorably. The striking effect was the rapidity with which phagocytosis of the bacilli set in and the growth of the bacilli became arrested, to which circumstances we attribute the recovery of the animal. A certain degree of phagocytosis occurs in human cases of influenzal meningitis, but the great number of bacilli remain outside of cells and are readily cultivated at all periods of the disease.

In the next experiment, the interval between infection and treatment was lengthened to six hours, and in the next, to nine and one half hours. Both terminated successfully. The interval was now increased to twenty-four hours, resulting, as will be seen, in perfect recovery.

Experiment XI.—Macacus rhesus. Usual inoculation, and twenty-four hours later, at which time the animal was very ill, 4 c.c. of serum injected. The highly turbid fluid withdrawn contained many bacilli and leucocytes, the former practically all outside of cells. Abundant cultures. The next day (forty-eight hours after inoculation), the monkey was greatly improved; 3 c.c. of serum injected. The fluid withdrawn was turbid and showed few bacilli, but they were profuse in culture. Third day, animal lively; 3 c.c. of serum injected; spinal fluid less turbid; very few bacilli; three colonies in culture. Fourth day, spinal fluid clear. Recovery complete.

The interval has not, up to the present, been increased beyond twenty-four hours. Doubtless animals could be saved at still later periods, but in view of the fact that individual peculiarities play a part in monkeys as in human beings in determining the period of survival, which may be as brief as twenty-four to thirty-six hours, and which in monkeys does not exceed three to four days, while in human beings it may extend to six or seven days, we have concluded that the longer period employed is great enough to establish the value of a local serum therapy in the experimental influenzal form of meningitis. In view of the severe conditions surrounding

influenzal meningitis in human beings, it would seem desirable to apply the serum to the treatment of the spontaneous disease. If this should be done, then every effort should be directed to the making of the bacteriological diagnosis at the earliest possible moment, and the employment of a serum that has been prepared with virulent influenza bacilli and shows a high degree of opsonic value.

We have not discussed the mechanism of action of the immune serum. That it is not bacteriolytic has been stated; but whether its whole activity depends upon its opsonic content and value, we have not undertaken to ascertain. In this paper we have confined ourselves to the presentation of certain facts which lend a hope for the better solution of the problem surrounding the condition of influenzal meningitis in man.

We have made one experiment for the purpose of determining whether influenzal meningitis in monkeys is an infection or merely an intoxication. Thus, the usual quantity of culture of *Bacillus influenzæ,* killed at 56° C., was injected into the spinal canal of a rhesus monkey. The injection produced no marked symptoms; from which it was concluded that the fatal effects of influenzal meningitis in monkeys, as in man, depend upon multiplication of the influenza bacilli and the gradual intoxication thus produced.

CONCLUSIONS.

The injection of virulent cultures of *Bacillus influenzæ* into the subdural space of several species of lower monkeys is followed by the development of an acute inflammation of the meninges, corresponding in clinical, bacteriological, and pathological effects with influenzal cerebrospinal meningitis in human beings.

Experimental influenzal meningitis in the monkey is a lethal disease which terminates fatally in from thirty-six hours to four days after the inoculation.

The injected influenza bacilli produce their effects through multiplication in the course of which they penetrate from the subdural space into the general blood current, from which they may be recovered during life and at autopsy, as is also true of the spontaneous form of influenzal meningitis in man.

By repeated injection, over a period of many months, of living virulent cultures of *Bacillus influenzæ* into the goat, an immune serum possessing moderate agglutinating and high opsonic power may be produced, which is capable, when injected into the subdural space, of arresting the progress of an experimental influenzal meningitis, and of bringing about recovery in monkeys thus affected.

As a result of the serum injections, the influenza bacilli in the meninges are more freely englobed by phagocytes, their number is reduced, their capacity of growth diminished, and the eruption into the blood arrested. Along with these effects go, hand in hand, cessation of the local inflammatory process and progressive amelioration of the symptoms of illness, to be followed usually by rapid restoration of health.

In view of the highly fatal character of influenzal meningitis in human beings, the employment of an immune serum by subdural injection is recommended. Undoubtedly it will be necessary to apply the serum early and by repeated injections, by means of lumbar puncture, to secure beneficial results. The early application will, in turn, be dependent upon prompt bacteriological diagnosis, which can be made, as a rule, by the immediate microscopical examination of the cerebrospinal fluid without the employment of cultural methods. When possible, the microscopical diagnosis should be confirmed by cultural tests.

ON THE FORMATION OF PRECIPITATES AFTER THE INTRAVENOUS INJECTION OF SALVARSAN.*

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During the past year there have appeared in the literature several reports of serious results—in a few cases even of death—following the intravenous injection of salvarsan. It was some time, however, before the chemical reaction of the salvarsan solution used was considered in connection with certain of these fatal cases. Fraenkel and Grouven (1), for instance, reported the death, within a few hours, of a patient who received intravenously 0.4 gram of salvarsan dissolved in fifteen cubic centimeters of water. These authors considered the symptoms following the injection to be those of arsenic poisoning, and concluded that death was due to a special hypersensitiveness of the patient to the drug. Ehrlich (2), commenting upon the case of Fraenkel and Grouven, agrees with them that the death of their patient was due to a preëxisting hypersensitiveness of the individual. He refers to other cases, in which, as in the case of Fraenkel and Grouven, there were advanced degenrative changes in the central nervous system, and in which there seemed to be a special susceptibility to the drug. Willige (3), however, thinks that death was due in the case of Fraenkel and Grouven to the use of a solution of too high concentration and suggests that only solutions of much lower concentration should be used intravenously.

H. E. Hering (4) first called attention to the dangers of using acid solutions of salvarsan. In experiments upon rabbits and dogs he determined the lethal dose of a one half per cent. solution. For rabbits he found it to be from four to five milligrams per kilo of body weight, and for dogs, from ten to twenty milligrams per

*Received for publication, May 22, 1911.

kilo of body weight. From the fatal doses in rabbits he calculated an average fatal dose for man, and came to the conclusion that the fatal result in the case of Fraenkel and Grouven may have been due to the chemical reaction of the acid solution. He warned against the use of the acid solution intravenously in man.

Auer (5), in a paper which is in press, has shown, however, that the concentration of the acid solution of salvarsan is a definite factor in the survival of an animal, and that much larger doses of salvarsan can be given in a dilute solution, without fatal results, than in a more concentrated solution. Auer's experiments were carried out upon rabbits. His results from the use of a one half per cent. solution of acid salvarsan agree with those of Hering already mentioned; *i. e.*. he found that very small doses of this solution were fatal. However, when a one tenth per cent. solution was used, as much as twenty-two milligrams per kilo of body weight produced no apparent harmful effect. There was not even a fall of blood pressure during the injection. Moreover, after a period of from two to four weeks, several of these animals were reinjected (in a few cases two or three times) with doses of twenty milligrams each, per kilo of body weight, using a one tenth per cent. solution. No injurious effect was ever seen.

The cause of the fatal results following the intravenous injection of acid solutions of salvarsan has been discussed in the literature. Some writers have considered that the acidity was the real cause. Most writers at present agree, however, that the amount of acid present in a lethal dose is too small to produce death.

For some time it has been a well known fact that a precipitate is formed upon the addition of an acid solution of salvarsan to blood *in vitro*. Miessner (6) showed that the acid solution produces a heavy precipitate when added to defibrinated blood or to bovine or horse serum. If an alkaline solution of salvarsan was used, he failed to obtain this precipitate. Schottmüller (7) has reported the same results from mixtures *in vitro* of acid and of alkaline solutions of salvarsan and blood plasma. Both Miessner and Schottmüller suggest that these precipitates are formed within the body[1]

[1] Michaelis (8) made a similar statement before either Miessner or Schottmüller.

as well as in the test-tube, and that the formation of a precipitate may have been the cause of death in certain of the cases which have been reported in man following the intravenous injection of the acid solution of salvarsan. These were, however, as stated, mere suggestions and no experiments were made by these or other writers to determine whether a precipitate is actually formed within the animal body. Such experiments have now been made, and the results are given in this paper.

METHOD.

Dogs and rabbits were used in these experiments. The dogs were etherized by means of the intratracheal insufflation method of Meltzer and Auer (9), and cannulas were inserted into a jugular vein and a femoral artery. Moderate ether anesthesia was maintained usually throughout the injection of salvarsan. The salvarsan was infused from a burette into the jugular vein. A sample of blood was drawn from the femoral artery before starting the infusion of salvarsan (sample 1—control), and another (sample 2) at the end of it. Then by means of a pipette inserted through the ventricular wall, samples were taken from the left ventricle (sample 3), and from the right ventricle (sample 4). One or more lobes of lung were snipped off and as much blood as possible was squeezed out (sample 5). To prevent coagulation, each of these samples of blood was collected in a small amount of a 0.03 per cent. solution of hirudin in saline solution. The samples were examined by spreading a film of blood over a glass plate, when the presence or absence of a precipitate could be made out either with the naked eye or with a fairly strong hand lens.

Most of the rabbits were treated in essentially the same way as the dogs. They were etherized, tracheotomized, and cannulas were inserted in one jugular vein and in one carotid artery. The salvarsan was injected into the jugular vein from a burette and blood samples were drawn from the carotid artery. During the injection, ether was discontinued in most cases. Then at the end of the salvarsan injection, or sometimes shortly before, ether was again used and the chest opened under artificial respiration to obtain samples of blood from the heart. Artificial respiration was maintained

in order to prevent stoppage of the heart during the taking of the blood samples, since it was desired to obtain the blood in a condition as nearly normal as possible. In all cases, the heart continued to beat efficiently until artificial respiration was discontinued. The method of taking samples of blood corresponded exactly to those described above for dogs.

In a number of experiments, portions of the control blood (sample 1) were mixed *in vitro* with different quantities of either acid or alkaline salvarsan solutions of different concentrations.

The acid and alkaline solutions of salvarsan were used in both dogs and rabbits, and the dosage in practically all cases was, in proportion to body weight, as large as that usually employed in man or even larger.

The acid solutions were prepared by grinding up the salvarsan in a mortar with a 0.9 per cent. solution of sodium chlorid which was added slowly in small amounts until the required dilution of salvarsan was obtained.

The alkaline solutions were prepared by adding to an acid solution of salvarsan in saline solution, normal sodium hydroxid (4 per cent.) in the proportion of one cubic centimeter of normal sodium hydrate to 0.1 gram of salvarsan. This is the minimum amount of alkali which will redissolve the precipitate that is thrown down when the acid solution approaches the neutral point. The acid and alkaline solutions were perfectly clear when injected. They were made up shortly before being used.

The concentrations of acid salvarsan used were, in dogs 1:200 (one half per cent.), and in rabbits 1:500 (one fifth per cent.). Of the alkaline solutions, the concentrations used were, in dogs 1:200 and 1:500, in rabbits 1:500. It might be mentioned in this connection that the solution usually employed in man for intravenous injections is a 1:500 solution.

EXPERIMENTAL RESULTS.

THE EFFECTS OF ACID SOLUTIONS OF SALVARSAN IN DOGS.

It will be recalled that the concentration of the acid solutions used in dogs was 0.5 per cent. By referring to table I, it will be seen

that seven experiments were performed. The dose of salvarsan varied between fourteen milligrams and thirty-three milligrams per kilo of body weight. There was also some variation in the rate of injection, as indicated in column 6.

TABLE I.

Acid Solutions of Salvarsan Given Intravenously to Dogs.

Number of experiment.	Body weight in gm.	Concentration of salvarsan used.	Amount of salvarsan injected in c.c.	Amount of salvarsan per kilo of body weight in mgm.	Duration of injection in minutes.	Blood samples examined for precipitate.			
						Arterial at end of salvarsan injection.	Direct from right ventricle.	From lungs and pulmonary artery.	Direct from left ventricle.
2	4300	1:200	17½	20	18	No ppt.			
3	5600	1:200	23	20	7	No ppt.			
4	4600	1:200	13	14	2½	Definite moderate ppt.			
5	4400	1:200	26	30	12	Definite moderate ppt.	Definite moderate ppt.	At least as much ppt. as from right ventricle.	No ppt. (11 minutes after injection).
6	4300	1:200	23	26	5	No ppt.	Large amount of ppt.	At least as much ppt. as from right ventricle.	Trace of ppt. (?).
7	4800	1:200	32	33	6¼	No ppt.	Fairly heavy ppt.	About same as from right ventricle.	Very slight ppt.; much less than from right ventricle.
14	5100	1:200	22	21	4		Heavy ppt.	About same as from right ventricle.	No ppt.

In six experiments, samples of blood were taken from the femoral artery at the end of the injection of salvarsan. In four of these there was no precipitate whatever to be seen. Two contained a moderate but definite precipitate.

In four of the seven experiments, samples were obtained from the right and left ventricles and from the lungs. In all four samples from the right ventricle there was a perfectly definite precipitate present in the blood. In three of the four cases, the right ventricle blood contained a *heavy* precipitate.

In each of the four experiments, a precipitate was obtained from the vessels of the lungs in at least as large a proportion as was found in the blood from the right ventricle. In the four experi-

ments in which samples of blood were taken from the left ventricle, two contained no precipitate at all, one a doubtful trace, and one a very slight amount of precipitate—markedly less than was found in the blood from the right ventricle.

In these experiments, then, we find that there was in each sample studied a precipitate in the blood from the right ventricle and also in the blood from the lungs; but that in only two out of six cases was there any precipitate in the arterial blood, and that in only two out of four was there a precipitate in the blood from the left ventricle, one of these two being doubtful. In other words, the precipitate was much less frequent in the arterial blood than in the blood from the right ventricle and lungs.

Portions of control (normal) blood were mixed with some of the 0.5 per cent. acid solution of salvarsan in the test-tube or on a glass plate. The proportions varied from one part of salvarsan to thirteen of control blood, to one part of salvarsan to fifty-four parts of blood. In all cases a precipitate was formed, concerning the existence of which there was no question even to the naked eye.

THE EFFECTS OF ACID SOLUTIONS OF SALVARSAN IN RABBITS.

Nine experiments were performed. The concentration of the salvarsan solutions used was 1 :500. It will be seen from table II that the dose of salvarsan injected into rabbits (varying from nineteen milligrams to seventy-one milligrams per kilo of body weight) was relatively larger than that given to dogs.

In five of the nine experiments, samples of blood were obtained from the carotid artery at the end of the injection of salvarsan. In not a single case was any precipitate found, though in one of these animals the dose of the drug was seventy-one milligrams per kilo of body weight.

In eight of the nine experiments, samples of blood were obtained from the right ventricle. In six of these, there was a definite precipitate present which varied in amount in the different animals from "moderate" to "heavy." In two, no definite precipitate could be found. In one of these two (experiment 9, table II), the injection was made rather slowly—fifteen cubic centimeters in ten

TABLE II.

Acid Solutions of Salvarsan Given Intravenously to Rabbits.

Number of experiment.	Body weight in gm.	Concentration of salvarsan used.	Amount of salvarsan injected in c.c.	Amount of salvarsan per kilo of body weight in mgm.	Duration of injection in minutes.	Blood samples examined for precipitate.				Mixtures of blood and salvarsan *in vitro*.
						Arterial at end of salvarsan injection.	Direct from right ventricle.	From lungs and pulmonary artery.	Direct from left ventricle.	
2	1650	1:500	32	40	14	No ppt.				
3	1400	1:500	50	71	27	No ppt.	Heavy ppt.		Possibly a trace of ppt. (?)	
4	1600	1:500	15	19	5		Heavy ppt.	Heavy ppt.	No ppt.	
6	1400	1:500	15	21	3		Distinct finely divided ppt.		Possibly a trace of ppt. (?)	
7	1740	1:500	16	19	4		Distinct finely divided ppt.	No ppt.	No ppt.	
9	1670	1:500	15	19	10		No definite ppt.	No definite ppt.	No ppt.	
17	1610	1:500	25	31	13½	No ppt.	No definite ppt.	Large amount of ppt.	No ppt.	6 parts blood + 1 part acid salvarsan 1:500 = heavy ppt. 4 parts blood + 1 part alkaline salvarsan 1:200 = no ppt.
18	1460	1:500	25	35	11	No ppt.	Moderate ppt.	Considerable ppt.	No ppt.	1 part blood + 1 part acid salvarsan 1:500 = heavy ppt. 1 part blood + 1½ parts alkaline salvarsan 1:200 = no ppt.
19	1490	1:500	25	35	9	No ppt.	Large amount of ppt.	Moderate amount of ppt.	No ppt.	

minutes. In the other experiment in which no precipitate was found in the blood of the right ventricle (experiment 17, table II), about ten minutes elapsed between the end of the injection and the taking of the sample. In this case, a large amount of precipitate could be demonstrated in the blood from the pulmonary vessels. It is possible, therefore, that the precipitate had passed on out of the right ventricle in this instance. At any rate in only one of the nine animals was no precipitate found in the blood from the right ventricle or the lungs.

In six of the nine experiments, samples of blood were obtained from the lung. In four of the six, a definite precipitate was found. The amount of precipitate present was at least as great as that contained in the blood from the right ventricle.

In eight of the nine experiments, samples of blood were obtained from the left ventricle. In six of the eight samples, there was no precipitate whatever. In the other two, there may have been a trace, though this was doubtful.

Stated briefly, the results of the injection of acid salvarsan solutions intravenously in rabbits were as follows: The arterial blood (carotid) at the end of the injection contained in no case a precipitate; blood from the left ventricle almost never; while blood from the right ventricle and lungs contained in nearly all cases a definite, and in some cases a very heavy precipitate.

It should be mentioned that in the right ventricle of one animal (No. 19, table II) at autopsy there were large masses of precipitate mixed with clotted blood clinging to the chordæ tendinæ. These masses occupied one half or more of the cavity of the ventricle. Similar masses were found in another rabbit and in one dog of this series.

ANIMALS SURVIVING THE INJECTIONS OF ACID SOLUTIONS OF SALVARSAN.

Experiments were performed upon four rabbits to observe the after-effects, if any, of the injections. These rabbits received doses of acid solutions of salvarsan which would surely have produced a precipitate within the blood-vessels in the experiments already described. The injections were made into the ear vein without any

operative procedure and the animals were allowed to live. Two of the four rabbits received twenty milligrams of acid salvarsan per kilo, and another received twenty-five milligrams per kilo. In all three, a one fifth per cent. solution of salvarsan was used. The fourth rabbit received ten milligrams per kilo of body weight of a one half per cent. solution of acid salvarsan. No ill effects whatever were observed in any of the four rabbits. Two were killed after forty and forty-one days respectively, and two after six days. Nothing abnormal was found at autopsy in any of them. It may be recalled here that the experiments of Auer already mentioned showed also that large doses of acid solutions of salvarsan could be given intravenously without any harmful effect if introduced in a dilute form, and that these large doses might even be repeated several times in the same animal with impunity.

THE EFFECTS OF ALKALINE SOLUTIONS OF SALVARSAN IN DOGS.

Injections of alkaline salvarsan solutions were made into six dogs (see table III). The concentrations of salvarsan used were 1:200 and 1:500.

Blood was obtained from the carotid arteries of six animals, from the right ventricles of seven, from the lungs of three, and from the left ventricles of seven; but in none of the samples was a precipitate found. Moreover, these alkaline solutions were injected at a rate considerably in excess of that employed with the acid solutions already described, a procedure which should have favored the appearance of a precipitate, if there was a tendency to precipitate formation.

As indicated in table III, mixtures of control blood and salvarsan solutions of different concentrations and chemical reactions were made. Not all these tests are recorded here. It will be seen in experiment 13 that a mixture of one part of a 1:500 acid solution of salvarsan to four parts of control blood gave a heavy precipitate, while one part of blood and one part of a one per cent. solution of alkaline salvarsan—a solution five times as strong as the former—gave no precipitate even upon standing. After some time, hemolysis took place in this latter mixture.

TABLE III.

Alkaline Solutions of Salvarsan Given Intravenously to Dogs.

Number of experiment.	Body weight in gm.	Concentration of salvarsan used.	Amount of salvarsan injected in c.c.	Amount of salvarsan per kilo of body weight in mgm.	Duration of injection in minutes.	Blood samples examined for precipitate.				Mixtures of blood and salvarsan *in vitro*.
						Arterial at end of salvarsan injection.	Direct from right ventricle.	Direct from lungs and pulmonary artery.	Direct from left ventricle.	
8	4550	1:500	53	23	3½	No ppt.	No ppt.	No ppt.	No ppt.	1 part blood + 1 part 1:500 alkaline salvarsan = no ppt.
9	4400	1:200	31	35	8	No ppt.	No ppt.	No ppt.	No ppt.	1 part blood + 1 part 1:200 alkaline salvarsan = no ppt.
										3 parts blood + 1 part 1:200 acid salvarsan = heavy ppt.
10	3700	1:500	53	28	4	No ppt.	No ppt.		No ppt.	1 part blood + 1 part 1:500 alkaline salvarsan = no ppt.
										1 part blood + 1 part 1:100 alkaline salvarsan = no ppt.
11	4400	1:500	80	36	7	No ppt.				1 part blood + 1 part 1:500 alkaline salvarsan = no ppt.
12	6400	1:200	40	31	3½	No ppt.				1 part blood + 2 parts 1:200 alkaline salvarsan = no ppt.
13	3700	1:200	24	32	2½	No ppt.				1 part blood + 1 part 1:100 alkaline salvarsan = no ppt.
										4 parts blood + 1 part 1:100 acid salvarsan = heavy ppt.
										4 parts blood + 1 part 1:500 acid salvarsan = heavy ppt.

TABLE IV.

Alkaline Solutions of Salvarsan Given Intravenously to Rabbits.

Number of experiment.	Body weight in gm.	Concentration of salvarsan used.	Amount of salvarsan injected in c.c.	Amount of salvarsan per kilo of body weight in mgm.	Duration of injection in minutes.	Blood samples examined for precipitate.				Mixtures of blood and salvarsan *in vitro*.
						Arterial at end of salvarsan injection.	Direct from right ventricle.	From lungs and pulmonary artery.	Direct from left ventricle.	
10	1430	1:500	15	21	7	No ppt.	No ppt.	No ppt.	No ppt.	1 part blood + 1 part alkaline salvarsan 1:500 = no ppt.
11	1320	1:500	15½	24	8½	No ppt.	No ppt.	No ppt.	No ppt.	1 part blood + 1 part alkaline salvarsan 1:500 = no ppt. 1 part blood + 1 part alkaline salvarsan 1:200 = no ppt. 2 parts blood + 1 part alkaline salvarsan 1:100 = no ppt.
12	1050	1:500	25	50	12		No ppt.		No ppt.	1 part blood + 1 part alkaline salvarsan 1:500 = no ppt. 1 part blood + 1 part alkaline salvarsan 1:100 = no ppt.
13	1350	1:500	22	34	9½	No ppt.	No ppt.		No ppt.	1 part blood + 1 part alkaline salvarsan 1:100 = no ppt.
14	1130	1.500	30	54	9	No ppt.	No ppt.		No ppt.	1 part blood + 1 part alkaline salvarsan 1:100 = no ppt.
15	1070	1:500	32	64	10	No ppt.	No ppt.		No ppt.	1 part blood + 1 part alkaline salvarsan 1:200 = no ppt.
16	1510	1:500	32	43	10	No ppt.	No ppt.	No ppt.	No ppt.	1 part blood + 1 part alkaline salvarsan 1:500 = no ppt.

THE EFFECTS OF ALKALINE SOLUTIONS OF SALVARSAN IN RABBITS.

Seven rabbits were injected with alkaline solutions of salvarsan of a concentration of 1:500 (see table IV). Here again, as in the case of the rabbits which received injections of acid salvarsan, the dosage given was large—from twenty-one to sixty-four milligrams per kilo of body weight. The rate of injection was between two and three cubic centimeters per minute.

After the injection of salvarsan, samples of blood were obtained from the carotid arteries of six animals, from the right ventricles of seven, from the lungs of three, and from the left ventricles of seven; but in none of these was a precipitate found. We see, therefore, that in rabbits as well as in dogs no precipitate was found, even after injections of fairly large doses of alkaline solutions of salvarsan.

Mixtures of control rabbit blood and alkaline salvarsan solutions of concentrations of 1:100, 1:200, 1:500 were also examined *in vitro* for precipitate, but in no case was any found, even though equal parts of a one per cent. solution of salvarsan and blood were mixed.

Usually after standing for some time, a mixture of control blood and an equal amount of one half per cent. or 1 per cent. alkaline solution of salvarsan showed hemolysis.

SUMMARY AND DISCUSSION.

The results of these experiments are definite. There is, in the first place, a very striking difference with regard to precipitate formation between the acid and alkaline solutions of salvarsan when injected intravenously. Intravenous injections of alkaline solutions of salvarsan produce no precipitate in the blood, while injections of the acid solution nearly always give a precipitate. Furthermore, after injections of the acid solution, there is a striking difference between the blood from the right side of the heart and that from the left side. At the end of injections of an acid solution of salvarsan, a precipitate was seldom present in the arterial blood. Blood taken from the left ventricle at this time (at autopsy) also showed no precipitate in a large majority of cases; in eight experi-

ments there was no precipitate, in three a doubtful trace of precipitate, and in one a definite small amount. On the other hand, blood obtained from the right ventricle and the lungs showed a very different condition. In ten out of twelve animals (rabbits and dogs), blood from the right ventricle contained a definite precipitate, and in a number of these cases the amount of precipitate was large. Blood squeezed from the lungs showed in eight out of ten cases at least as much precipitate as was found in the blood from the right ventricle.

The results of injections of alkaline solutions of salvarsan, as pointed out before, are quite different from those produced by the acid solutions. In thirteen experiments upon dogs and rabbits, no trace of a precipitate was found in the arterial blood, the blood from the left ventricle, the right ventricle, or the lungs.

There is no apparent difference in the process of precipitate formation whether salvarsan solutions and the blood are mixed *in vivo* or *in vitro*. In both mixtures the acid solutions produce a precipitate, while the alkaline solutions of salvarsan do not.

These experiments have demonstrated the fact that a precipitate is present in the blood after an injection of an acid solution of salvarsan. One would expect that such a precipitate, consisting as it usually does of rather coarse particles, would, if brought to the medulla, cause immediate death by producing emboli. However, the freedom from such occurrences may be explained by the fact that the precipitate, which is abundantly present in the right ventricle, is only rarely seen in blood taken from the carotid or femoral arteries or even from the left ventricle itself. The fact itself, however, is quite difficult to interpret. It might perhaps be assumed that the precipitate is filtered out during its passage through the lung capillaries. If this is the case, we might expect intravenous injections of salvarsan to produce embolism in the pulmonary vessels with consequent fatal results.

As a matter of fact, we have in the recent literature an instance which seems to point to such a result. Miessner (6) tried the effects of salvarsan in cattle which had foot and mouth disease. He used at first the acid solution, and though the dose was small, seven milligrams per kilo of body weight, all the animals (four) died in

from ten hours to two days after the injection. They all showed labored respiration during or soon after the injection of salvarsan.

He then decreased the dose to five milligrams per kilo of body weight, and repeated the experiments. He used also normal animals as controls upon those which had the foot and mouth disease. Both the sick and normal (control) animals showed labored respiration. One died after four days. At autopsy all organs except the lungs appeared to be normal.

The lungs presented the following appearance: There were grayish yellow spots scattered irregularly over the surface. On the cut surface these were seen as grayish yellow spots the size of a pea, which appeared in groups and which sometimes filled a lobule completely. Other spots were surrounded by a small area of dark red lung parenchyma. The affected portion contained no air and felt solid. In adjacent parts the tissue seemed normal. A microscopic examination showed that the larger and smaller pulmonary arteries were filled with uniform, homogeneous, yellow masses. About the vessels there was a serous exudate.

In brief, the changes seen indicated, he believed, that there was a thrombosis of the blood-vessels with inflammatory exudative changes of the lung parenchyma. Miessner states that a similar pathological condition was found in a normal control animal that died.

He suggests that the acid solution of salvarsan might lead in man to a thrombosis of the pulmonary arteries. In support of this suggestion, he mentions a case reported to him by Ehrlich of a man who died following the injection of an acid solution. The lung picture in this case was somewhat similar to that which he had found in cattle.

It may be mentioned in passing that Miessner found that alkaline solutions of salvarsan were far less toxic than the acid solutions. Animals (cattle) which received in an alkaline solution 400 milligrams of salvarsan per kilo of body weight did not show the least symptom of disturbance.

In contrast to Miessner's results seem to stand my observations and those of Auer described in the introduction of this paper. Auer (5) found (in 8 rabbits) that no evident harmful effects

followed the injections of very large doses of the acid solution, if they were given in a highly diluted form (one tenth per cent.). In my own experiments, it was found that a one fifth per cent. acid solution (3 rabbits) and even a one half per cent. solution (1 rabbit) produced no ill effects. The experiments described in this paper make it certain that the doses of the acid solution given to these last mentioned four animals must have produced a precipitate in the right ventricle and in the lungs, and yet the animals survived and showed no symptoms whatever of disturbance following the injection.

This difference between our observations and those of Miessner might perhaps be explained by the assumption that the action of salvarsan in acid solution is more deleterious to cattle than to rabbits. Furthermore, Miessner seems to have injected the salvarsan in high concentrations. In one instance, in which figures are given, the drug was administered in a five per cent. solution. As mentioned before, Auer has shown the importance of the concentration. While in a one tenth per cent. solution twenty and thirty milligrams per kilo of body weight of the acid solution may be injected with impunity, even six or seven milligrams per kilo may prove rapidly fatal when injected in a one half per cent. solution.

Our own results, however, leave us with two puzzling questions: First, if the acid solution of salvarsan causes such a coarse precipitate in the right ventricle and in the lungs, how does it happen that this precipitate does not bring about the death of the animal? Second, what is the real cause of the remarkable fact that this precipitate does not pass over into the arterial side of the circulation? Does the precipitate undergo a profound chemical or mechanical change while it passes through the lung capillaries? In future investigations we may try to answer these interesting questions. For the present, it is necessary to be content with the establishment of the bare facts as they are presented in the conclusions.

CONCLUSIONS.

Acid solutions of salvarsan, injected intravenously into dogs and rabbits, even in the concentrations used in man, produce a precipitate in the blood stream.

This precipitate, which is easily seen and which is abundantly present in the blood from the right ventricle and lungs, can seldom be demonstrated in blood from the left ventricle and arteries.

Intravenous injections of salvarsan in doses which surely produce a precipitate in the blood stream, are not, at least in rabbits, necessarily fatal to the animal.

Alkaline solutions of salvarsan, even in strong concentration, never produce a precipitate when injected intravenously.

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